OPTIMIZATION OF MICROALGAE'S CULTURE CONDITIONS FOR CO_2 FIXATION, BIOMASS PRODUCTIVITY AND WASTEWATER TREATMENT.

BY

MOHAMMED KAZEEM AYODEJI

A Thesis Presented to the

DEANSHIP OF GRADUATE STUDIES

KING FAHD UNIVERSITY OF PETROLEUM & MINERALS

DHAHRAN, SAUDI ARABIA

In Partial Fulfillment of the
Requirements for the Degree of

MASTER OF SCIENCE

In

CHEMICAL ENGINEERING

APRIL 2018

APPROVAL PAGE

KING FAHD UNIVERSITY OF PETROLEUM & MINERALS

DHAHRAN- 31261, SAUDI ARABIA

DEANSHIP OF GRADUATE STUDIES

This thesis, written by **Mohammed Kazeem Ayodeji** under the direction his thesis advisor and approved by his thesis committee, has been presented and accepted by the Dean of Graduate Studies, in partial fulfillment of the requirements for the degree of Masters of Science.

Dr. Mohammed Saleh Ba-Shammakh
Department Chairman

Dr. Salam A. Zummo
Dean of Graduate Studies

26/4/2018
Date

Shaikh Abdur Razzak

Dr. Shaikh Abdur Razzak
(Advisor)

Mozahar Hossain

Dr. Mozahar Hossain
(Co-Advisor)

Dr. Mohammed Saleh Ba-Shammakh
(Member)

Dr. Mamdouh Al-Harthi
(Member)

Dr. Wasif Farooq
(Member)

This research is dedicated to my beloved wife Kareema Mohammad and our Daughter Qasima Mohammad

ACKNOWLEDGEMENT

All thanks and praises be to Allah (SWT), the most beneficent, the most merciful and glorification are due to Him who in his infinite mercy spared me to this stage of my life, for giving me the opportunity to witness this unique and historical achievement in my life and for elevating me to my present academic status. May the peace and blessing of Allah be upon His messenger Muhammad bin Abdullah (SAW), his household, his companions and his followers.

My sincere and infinite gratitude goes to Chemical Engineering Department for adding layers of knowledge to me and to the Department chairman.

I thank the funding agent, NSTIP and KACST for making this project possible.

My special gratitude goes as always to my Advisor, Dr. Shaikh Abdur Razzak and to my Co-Advisor Dr. Mohammed Mozahar Hossain whose expert advice on countless point of context, encouragement and support made this research a successful one.

My sincere appreciation goes to my committee members Dr. Mohammed Saleh Ba-Shammakh, Dr. Mamdouh Ahmed Al-Harthi, and Dr. Wasif Farooq for ensuring that the thesis ended well.

My special gratitude also goes to all the academic, non-academic and supporting staff of Chemical Engineering Department for contributing in one way or the other and for making my stay in the University a memorable and educative one.

My list of acknowledgements would not be complete without mentioning the immense contribution of Dr. Zaki Hossain from university of Bahrain on all my articles with regards to this thesis. May Allah bless you and relieve you of your worries.

My special gratitude also goes to the Chemical Engineering laboratory technicians Mr. Amanullah Rahamathullah and Mr. Mohammed Abdelaziz for their kind assistance throughout out the experiment.

My unreserve thanks go to my loving parents for bringing me up, training me accordingly and always helping me with prayers from my childhood to this level, May Allah in His infinite mercy grants them Al-Jannatul Firdausi (Amin).

My sincere appreciation goes to my all my family members, my wife, my daughter, my brother and sisters, Mohammed Kareemah, Mohammed Qasima, Mohammed Abubakir, Mohammed Zeenat, Mohammed Azeezat. Thank you very much for everything you have done.

Lastly, thanks to my friends and colleagues most especially Dr. Sagir Adamu, Musa Najimu, Taiwo Saheed and all the Nigerian Community at KFUPM (NCUPM) and to others who are not mentioned, you are not forgotten. Thanks for your support throughout the program at KFUPM. I will always remember you, you are always in my heart and I love you all.

Finally, I will like to extend my appreciation to all the final year students in Chemical Engineering Department wishing you all the best in life.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF MINITAB SEQUENCE

LIST OF ABBREVIATIONS

T_L	Lower bound of culturing temperature (^{0}C)
T_H	Upper bound of culturing temperature (^{0}C)
N_L	Lower bound of nutrient ratio (i.e. High phosphate concentration)
N_H	Upper bound of nutrient ratio (i.e. Low phosphate concentration)
C_L	Lower bound of CO_2 concentration (%)
C_H	Upper bound of CO_2 concentration (%)
-α, α	Extreme values/corner points
0	Center points
-1, +1	Low and high values respectively
S	Set of both equalities
x_1	Coded values for the carbon dioxide concentration
x_2	Coded values for the nutrient ratio
x_3	Coded values for the culturing temperature
xn	Set of pareto solution
CCD	Central composite design
BBM	Bold basal medium
MBBM	Modified bold basal medium
CCS	Carbon capture and storage
OD_{690}	Optical density at 690nm

SG	Specific growth rate
(P, g_{dw} L^{-1} d^{-1})	Productivity
BP	Biomass productivity
(R_c, g_{CO2} L^{-1} d^{-1})	CO_2 fixation rate
CO_2 Fix.	CO_2 fixation rate
LSR	Least squares regression
X_i	Biomass dry weight at the beginning of the cultivation
X_f	Biomass dry weight at the current day of the cultivation
t_i	Initial time (culturing days)
t_f	Current time (culturing days)
$\frac{M_{CO2}}{M_C}$	Ratio of molecular weight of Carbondioxide to Carbon
Cv.	*Chlorella vulgaris*
Ck.	*Chlorella kessleri*
Cp.	*Chlorella prototheocoides*
Neo O	*Neo oleoabundans*

ABSTRACT

Full Name	:	Mohammed Kazeem Ayodeji
Thesis Title	:	Optimization of Microalgae's Culture Conditions for CO_2 Fixation, Biomass Productivity and Wastewater Treatment.
Major Field	:	Chemical Engineering
Date of Degree	:	December 2017

Microalgae based biofuels have been reported as an attractive alternative for fossil fuel. However, producing biofuel from microalgae still not economic viable. Therefore, it integration with other applications like CO_2 capture and wastewater treatment would reduce the cost of production. Nevertheless, producing biofuel from microalgae strongly depends on the lipid productivity of the microalgae which in turn depends on the microalgae strain and culture conditions. However, the adeptness to optimize microalgae biomass productivity and CO_2 biofixation especially under the varying conditions is crucial for evaluating the profitability and sustainability of their cultivation at large scale for biofuel industries. Therefore, this study first investigates the effect of CO_2 concentration on microalgae growth rate, atmospheric CO_2 capture rate and efficiency of wastewater treatment. Four different microalgae strains, *Chlorella vulgaris, Chlorella kessleri, Chlorella prototheocoides and Neo oleoabundans,* were studied to ascertain the most advantageous regarding the preferred applications. However, the results showed that *C. vulgaris* have the highest CO_2 uptake rate of 150_mg/L/day. Multi-objective optimization of some microalgae's culturing parameters (i.e. CO_2 concentration, nutrient ratio and culturing temperature) was further carried out on biomass productivity and CO_2 fixation rate for *C. vulgaris*. Quadratic regression models were developed and employed to estimate the optimal sets of input factors for biomass productivity and CO_2 fixation rate operated in batch mode. The obtained

optimal values were 4% CO_2 concentration, 6:1 Nutrient (NP) ratio and 25^oC culturing temperature for 183_mg/L/day CO_2 fixation rate and 0.0785_g/L/day biomass productivity. Multi-objective optimization also was performed to maximize CO_2 fixation rate, wastewater treatment efficiency of the microalgae. The predicted optimal values of 4% CO_2, 7:1 NP ratio and 28^oC culturing temperature gave maximum CO_2 fixation rate of 127.7 ± 5.1 $mgL^{-1}day^{-1}$, nitrogen removal efficiency of 99.9 ± 7.7 % and phosphorus removal efficiency of 88.5 ± 3.6%. The maximum percentage error between the predicted and experimental values was less than 12% indicating a good agreement between the predicted and the actual data. Hence, these findings have the potential to be used in the design and scale-up of industrial microalgae cultivation with the aim of CO_2 fixation, wastewater treatment and generation of biomass for biofuel industries.

نبذة مختصرة

وقد تم الإبلاغ عن الوقود الحيوي القائم على الطحالب الدقيقة كبديل جذاب للوقود الأحفوري. ومع ذلك، فإن إنتاج الوقود الحيوي من الطحالب الدقيقة لا يزال غير قابل للاستمرار اقتصاديا. ولذلك، فإن التكامل مع التطبيقات الأخرى مثل التقاط ثاني أكسيد الكربون ومعالجة مياه الصرف الصحي من شأنه أن يقلل من تكلفة الإنتاج. ومع ذلك، فإن إنتاج الوقود الحيوي من الطحالب الدقيقة يعتمد بشدة على إنتاجية الدهون في الطحالب الدقيقة التي تعتمد بدورها على سلالة الطحالب الدقيقة والظروف الثقافية. ومع ذلك، فإن الكفاءة في تحسين إنتاج الكتلة الحيوية الطحالب الدقيقة وبيوفكساتيون CO_2 خاصة في ظل ظروف متفاوتة أمر بالغ الأهمية لتقييم ربحية واستدامة زراعة على نطاق واسع لصناعات الوقود الحيوي.
ولذلك، فإن هذه الدراسة تدرس أولا تأثير تركيز ثاني أكسيد الكربون على معدل نمو الطحالب الدقيقة، ومعدل التقاط ثاني أكسيد الكربون في الغلاف الجوي، وكفاءة معالجة مياه الصرف الصحي. تم دراسة أربع سلالات طحلبية مختلفة، كلوريلا فولغاريس، كلوريلا كيسليري، كلوريلا بروثوثوكيدس و نيو أوليوابوندانز للتأكد من الأكثر فائدة فيما يتعلق التطبيقات المفضلة. ومع ذلك، أظهرت النتائج أن C. فولغاريس لديها أعلى معدل امتصاص CO_2 من 150_L / mg / يوم.
كما تم إجراء تحسين متعدد الأهداف لبعض معلمات زراعة الطحالب الدقيقة (أي تركيز ثاني أكسيد الكربون ونسبة المغذيات ودرجة حرارة الزراعة) على إنتاجية الكتلة الحيوية ومعدل تثبيت ثاني أكسيد الكربون ل C. فولغاريس. تم تطوير نماذج الانحدار التربيعي واستخدامها لتقدير المجموعات المثلى لعوامل المدخلات لإنتاجية الكتلة الحيوية ومعدل تثبيت ثاني أكسيد الكربون الذي يتم تشغيله بطريقة الدفعات. وكانت القيم المثلى التي تم الحصول عليها هي 4٪ تركيز CO_2، 16 : نسبة المغذيات (نب) ودرجة حرارة زراعة oC25 ل 183_L / mg / يوم معدل تثبيت CO_2 و 0.0785_L / g / يوم إنتاج الكتلة الحيوية. ويمكن استخدام هذه النتائج في تصميم وتوسيع نطاق زراعة الطحالب الصناعية الصناعية بهدف تثبيت ثاني أكسيد الكربون وتوليد الكتلة الحيوية لصناعات الوقود الحيوي.

CHAPTER 1

INTRODUCTION

1.1. Background and Statement of Problem

The demand for world second most vital commodity, energy after water (Kevin, 2011) continue to increase as a result of increase in the world population growth, urbanization and industrialization. Fossil fuels remain one of the world major sources of energy. However, recent studies reiterated that continue utilization of fossil fuel can no longer be sustained due to rapid dwindling of our fossil fuel reserves (i.e. crude oil, coal etc.) and environmental problems linked to combustion of fossil fuel (Razzak *et al.*, 2013).

Fossil fuel combustion contributes largely to the emission of CO_2 in to the atmosphere which is one of the major greenhouse gasses known resulting to global warming. Kyoto protocol among others as an example of the international pacts in 1997 mooted global efforts to cut down the emission of CO_2. However, the global daily mean concentration of CO_2 was been reported to be above 400ppm for the first time in July 2016 as shown in Fig. 1-1. This rapid increase in the atmospheric CO_2 concentration could result to a derelict planet and irreversible phenomenon for the upcoming generations. At present, the society we live in is faced with the challenges of concomitantly increasing the supply of energy and reducing CO_2 emission. Conversely, due to the CO_2 emission level in the year 2000, about 50-80% global reduction in CO_2 emission is required to maintain 2 - 2.4^{O}C rise in global mean temperature (Zimmerman and Faris, 2011).

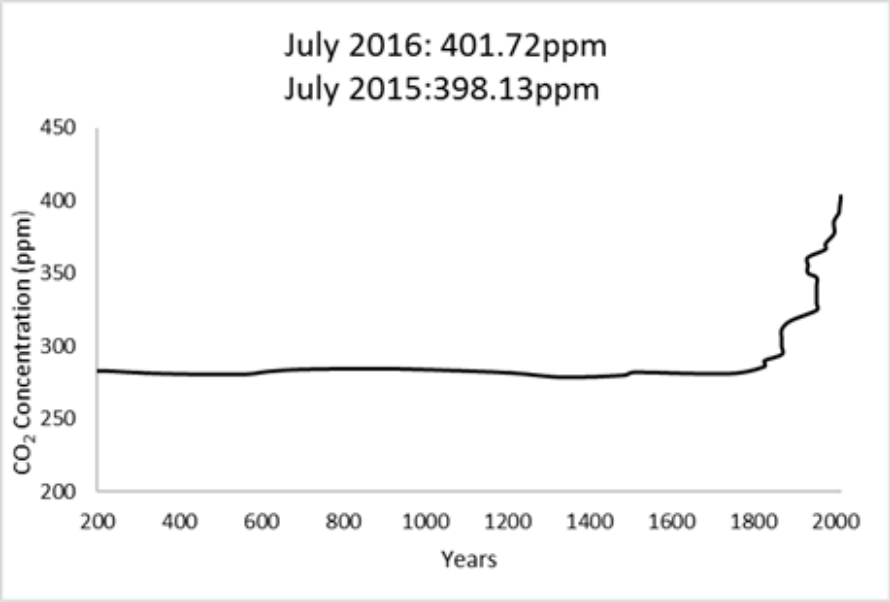


Fig. 1-1: Global trends in atmospheric Carbon dioxide concentration (Mauna, 2016)

1.2. Brief State of Art on Different Approaches

An approach initiated to reduce the emission of CO_2 in the atmosphere includes those that are based on the technologies like adsorption, cryogenic distillation, absorption and membrane separation which are being studied to capture carbon dioxide from units where they are emitted (Stepan *et al.*, 2002). Captured Carbon dioxide is then channeled and saved at high concentrations in geological formations (Kasiri *et al.*, 2015a). Since the capturing and storage technology are temporary solution, therefore, environment sustainability of the above mentioned methods became problem (Pires *et al.*, 2012). However, a biological method for capturing of CO_2 using microalgae became one of the best option since it's an Eco friendly process and does not need further disposition of the captured carbon dioxide (Cheng *et al.*, 2013).

Moreover, since it has been established that energy and transportation sector represents the major fraction of CO_2 emissions, so it makes sense if alternate sources of energy are provided for those

sectors. Renewable energy sources became the option that worth looking into. Examples of these sources include solar (which is thermal or electrical energy derived from the sun), wind (which is the energy derived from the power of moving air), geothermal (which is the energy derived from heat beneath the earth crust), hydropower (which represent the energy derived from the power of moving or flowing water) and biofuels. Biofuels as the name Implies are fuels derived from bio-origin which include bioethanol, biodiesel, bio hydrogen and biogas. Although the combustion of these biofuels also emit CO_2, but the growing biomass would capture the CO_2 and therefore leaves net zero CO_2 emission or even negative, consequently, the maintenance of CO_2 level in the atmosphere would be achieved (Rhodes & Keith, 2008; Mathews, 2008; Farrelly *et al.*, 2013).

Several biomasses including plants and agricultural wastes have been investigated for their potential use of biofuel production. However the research interest on the use of microalgae as a substitute for conventional biofuel feedstock continue to increase (Mata *et al.*, 2011) because they have no competitive use as food unlike the other plants. Also, they can be continuously cultivated and harvested all year round as they require no fertile land and little space for their cultivation therefore eliminating seasonal challenges that could be facing biofuels industry. Microalgae photosynthesis efficiency is about ten times higher than that of terrestrial plants exhibiting rapid growth rate thereby producing much higher valued biomass materials which can serve as a feedstock for varieties of sectors like animal or fish feed, human food, fertilizer, cosmetics and biofuels (Pires *et al.*, 2012). Also, they have the ability to used up nutrients from waste stream (i.e. wastewaters or emissions of flue gasses) which therefore help in reducing environmental effect and cost of their cultivation (Mata *et al.*, 2013).

Nevertheless, microalgae cultivation still presents high process costs. In addition, microalgae require large amounts of nutrients and water, this is a very important factor to be considered, which

has a high environmental impact (Lundguist *et al.*, 2010; Sialve *et al.*, 2009). To overcome these disadvantages, microalgae production can be joined alongside wastewater treatment.

Studies in wastewater treatment using microalgae started since the mid-1970s (Al Ketife et al., 2016). Photobioreactors provide a more sustainable option to the conventional biological nutrient removal method from the economical point of view. The conventional method requires energy during aeration and transfer of sludge from one tank to the other along the treatment scheme, and also additional chemical dosing with coagulants to arrive at the desired phosphorus removal (Maher et al., 2015). Wastewater treatment by microalgae is a process which results to a sustainable solution for combined nutrient and CO_2 obliteration, the footprint from this technique can be higher than second orders of magnitude to that from the conventional process (Judd et al., 2015). Ji et al., (2013) reported approximately 100% removal of phosphorus from tertiary municipal wastewater by using three different strains of microalgae, namely *Chlorella vulgaris*, *Scenedesmus obliquus* and *Ourococcus multisporus.* Novel technology for improving and optimising the process to aid the CO_2 fixation and wastewater treatment, is thus crucial.

In terms of removing nutrient by *Chlorella vulgaris*, a number of studies have been studied and reported as shown in Table 1-1

Table 1-1: % Removal of Nitrogen and Phosphorus by *Chlorella Vulgaris*

Nutrient ratio (NP)	Productivity (g/L/day)	Nitrogen removal efficiency (%)	Phosphorus removal efficiency (%)	References
3:1	0.234	67.2	97.8	(Ruiz-Martinez et al., 2012)
5:1	0.230	97	96	(Feng et al., 2011)
4:1	0.080	97.1	87.6	Present study
19:1	0.072	82.5	85.9	(Gao et al., 2015)
8:1	-	93	-	(Kapdan and Aslan, 2007)
6:1	0.070	97.2	88.2	Present study
2:1	0.062	99.5	24.4	Present study
21:1	0.054	70	100	(Silva-benavides and Torzillo, 2012)
7:1	-	86	70	(Lau et al., 1996)
10:1	0.003	78	89	(Choi and Lee, 2015)
20:1	0.002	84	81	(Choi and Lee, 2015)
193:1	-	44	84.2	(Boonchai et al., 2012)

It is important to note that optimization of several input parameters (e.g., strain of microalgae used, initial CO_2 concentration or organic carbon liquid load, light intensity, temperature, pH and feed water nutrient load) for several responses or outputs (e.g., the biomass productivity, CO_2 fixation rates, efficiencies in wastewater treatment) could be difficult due to a requirement of large number of experimental runs (Ghosh et al., 2015; Skorupskaite et al., 2015). For instance, the optimisation of three parameters in five levels would require 5^3 or 125 individual runs. To avoid such large number of runs, a statistical design of experiment (i.e. DOE) could be employed. This approach includes central composite design and Box-Behnken design, in which a considerable number of experiments is decreased. At the same time, synergetic effects among the various parameters are considered and thereby a true optimum set of conditions (Toktas et al., 2014) is achieved. Therefore, DOE could be an elegant and efficient approach for optimization study of any complex and multi-parameter system.

1.3. Research Goals

This study aimed to first investigate the effect of CO_2 concentration on the growth of *Chlorella vulgaris, Chlorella kessleri, Chlorella prototheocoides and Neo oleoabundans*) taking into account: (i) specific growth rate; (ii) biomass productivities; (iii) CO_2 fixation rate; and (iv) nitrogen and phosphorus uptake.

Secondly, a central composite response surface design was then employed to develop quadratic regression models for the specific growth rate, CO_2 biofixation rate and rate of nutrient removal by altering the initial nutrient concentration (i.e. nitrate and phosphate), culturing temperature and CO_2 concentration of *Chlorella vulgaris* cultivated in BBM media (batch process). Multiobjective optimization method was employed to determine the maximum specific growth rate, CO_2 biofixation rate and biomass productivity concurrently.

1.4. Structure of Thesis

- **Chapter 1**: This section introduces the reader to the general concept of the thesis objectives. It illustrates where the problem begins, gave brief state of art on different approaches to tackle the problem and was concluded with the study goals.

- **Chapter 2:** This chapter contain the detail literature review in the field of microalgae culturing. Highlights clearly the researches shortcomings to declare the thesis objectives.

- **Chapter 3:** The overall and specific thesis objectives were listed.

- **Chapter 4:** This section involves demonstration of the method for culturing microalgae, and the composition of culture media used. It also presents the detail description of the analytical methods in these investigations.

- **Chapter 5:** This chapter contains the investigations on the effect of CO_2 concentration on the rate of microalgae growth, CO_2 uptake and wastewater treatment efficiency.

- **Chapter 6:** Optimization study of the culture's conditions to enhance their CO_2 fixation rate and maximum biomass productivity. A statistical approach.

- **Chapter 7:** The concurrent investigation of CO_2 fixation rate, and wastewater treatment by microalgae.

- **Chapter 8:** Justification for accepting the regression models.

- **Chapter 9:** Conclusion and recommendations.

CHAPTER 2

LITERATURE REVIEW

2.1. Overview

In an approach to put microalgae as an agent for biological fixation of CO_2 in to competitive use with other carbon capture and storage (CCS) technologies, various investigations have been carried out to optimize the rate of CO_2 fixation by some strains. As it have been established in the literature, factors that remarkably affect the rate of biofixation of CO_2 by microalgae are light intensity, temperature, nutrients ratio, pH, photo bioreactors type, CO_2 concentration, microalgae species and gas flow rate (Cheng et al., 2013) and (Ho et al., 2012). The succeeding paragraphs shall discuss on the significance of each factors and the state of art on the attempt to optimize them to achieve higher microalgae specific growth rate and CO_2 biofixation.

2.2. Effect of Light Intensity on Microalgae Growth

Light demand for microalgae is one of the most significant factors to be dealt with, to be effective for microalgae grow; it needs to be supplied at the adequate wavelength, intensity and duration. Many facts from the literature acknowledged that excess intensity might result to photo oxidation and photo inhibition while little light intensity would retard the growth rate. Let look at how microalgae utilize the light energy.

Microalgae contains a number of pigment of which chlorophylls among others exhibit the ability to capture the light energy and convey it to reaction centre where it is then placed on the particular pigment pair situated in the photosystem (Carvalho et al., 2011). The reaction centre consist of 2 photosystem units which are photosystem 1 and 2. Photosystem 1 consists of mainly chlorophyll

and photosystem 2 consist of meaning amount of chlorophyll b. Both the photosystems convey e^- from H_2O to nicotinamide adenine dinucleotide phosphate. The heart centre of photosynthesis is the chlorophyll since it processes begins once photon from the light strike a molecule of the chlorophyll. The process of photosynthesis occurs in 2 phases namely; the first phase is set of reactions that can only occur in the presence of light and ATP to generate energy and intermediately uses NADPH to decrease power and the second phase constitute sets of reactions that occur in the dark or in the absence of light which include the Calvin cycle. Here, the intermediates obtained in the first phase would be utilized in the generation of glucose by reacting with Carbon dioxide. This would be illustrated in the diagrams below (Carvalho et al., 2011).

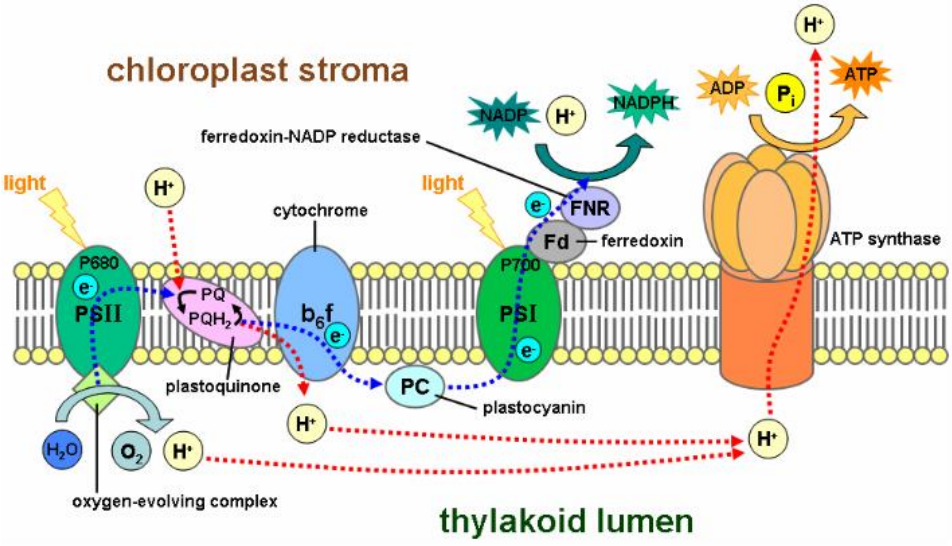


Fig. 2-1: Phase 1 (i.e. set of reactions that occur in the presence of light) (Kasiri, 2015)

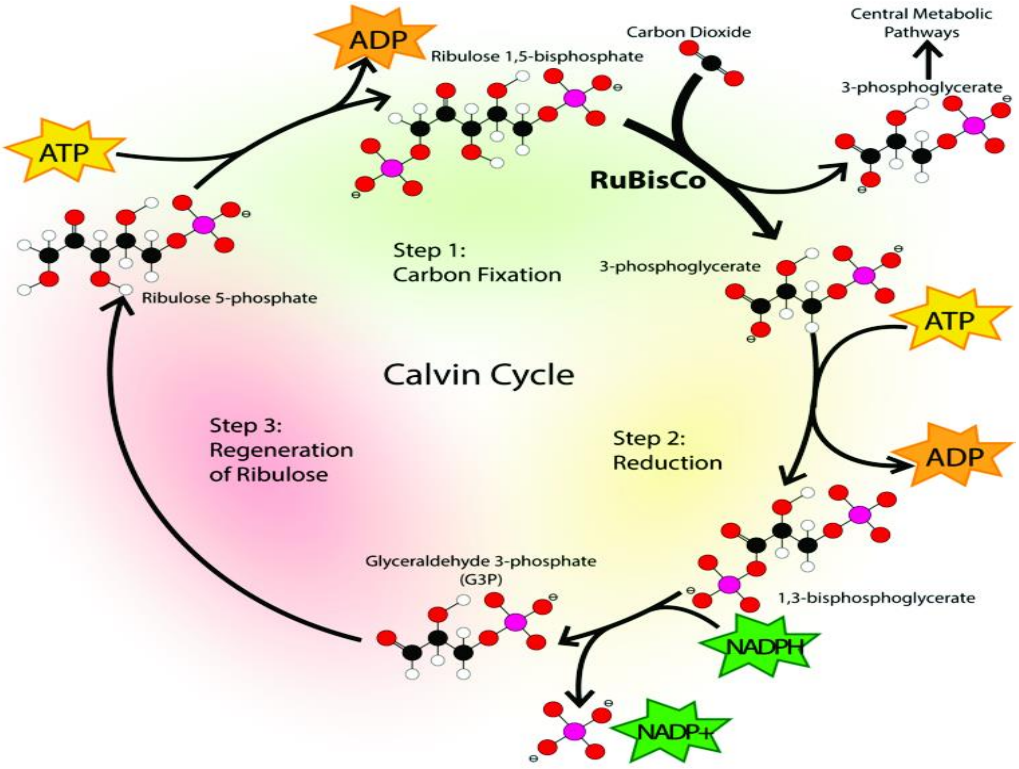


Fig. 2-2: Phase 2 (i.e. Calvin cycle and fixation of carbon) (Kasiri, 2015)

In view of the above, the significance of light intensity in the any growth which depend solely on photosynthesis can never enfeebled (LihaiFan *et al.,* 2007). Although several studies has been done as to determine the optimum light intensity to achieve the maximum growth rate possible for the particular strain because, as observed, increase in light intensity is said to increase the photosynthetic process which consequently increase the growth rate till it reaches saturation point where further increase in light intensity result to no increment and therefore, drastically reduces productivity because of the occurrence of a phenomenon called photo inhibition (LihaiFan *et al.,* 2007; Zheng *et al.,* 2011). Some few microalgae species have been reported in the literature, i.e. for *Scenedesmus* and *Chlorella Sp*., the saturation light intensity have been estimated to be 276 µmol m^{-2} s^{-1}, and for *chlorogleopsis sp.,* it optimum light intensity was observed to be 338 µmol m^{-2} s^{-1} (Zheng *et al.,* 2011).

However, studying only light effect on the growth rate could not satisfactorily predict the response of the outdoor large scale, especially when the nutrients lack some essential elements i.e. nitrogen starvation period. To correct this, (Cheng et al., 2013) Optimized the light intensity together with nutrients molar ratio (i.e. carbon to phosphorus, carbon to nitrogen, and carbon to magnesium) for Chlorella PY-ZU1. Carbon dioxide of 15% was used and adjustment of nutrients ratios made were from 0.17, 0.093 and 0.018 to 0.69, 0.096, and 0.0030 for N:C, P:C, and Mg:C respectively and as reported, these alterations gave about 125% increase in microalgae biomass production. Light intensity was then adjusted from 61 to 81 μmol m^{-2} s^{-1} and the specific growth rate was observed to have 99% increases.

Although all this studies are conducted at a specific temperature, and failed to realize that outdoor or sunlight intensity is inseparable with the temperature which means, the higher the light intensity, the higher the temperature and from literature, its established that, each microalgae strain show a particular growth rate correlation curve at a specific temperature (Carvalho *et al.,* 2011). Therefore, there is a need to study the effect of temperature simultaneously with the other factors reported.

2.3. Effect of CO_2 Concentration on Microalgae Growth

Just like the terrestrial plants, microalgae also utilize carbon dioxide to carryout photosynthesis in the presence of light. Broadly speaking, raising the carbon dioxide concentration enhances the process of photosynthesis in several microalgae strains since, higher availability of CO_2 increases it diffusion from bulk concentration to the active site of Rubisco as shown in **Fig. 2-2**. But it should be asserted that the increase in CO_2 concentration should not be above optimum level where the strains can endure (Sato *et al.,* 2003). Microalgae uses HCO_3^- which is formed from dissolution of CO_2 in the media as shown in the Eq. 2-1 (Moroney & Somanchi, 1999) below and excess

accumulation of these carbonic acid could make the media toxic of which it degree of effect vary from one strain to another (Razzak *et al.,* 2013). Although, most strain exhibit optimal growth at pH near neutral, however, some prefer acidic environment while others prefer alkaline medium (Kumar *et al.*, 2010).

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \qquad 2\text{-}1$$

The effect on CO_2 fixation and obtainable oil by *Nannochloropsis oculata* and *Tetraslmis chuii* have been studied by (Purba and Taharuddin, 2010) through the manipulation of CO_2 concentration from 3 – 9% and light intensity from 5 – 23 μmol m^{-2} s^{-1}. This study shows that both factors have significant effect on the CO_2 fixation rate. In the study, it was observed that increase in both CO_2 concentration and light intensity gives a corresponding increase in CO_2 biofixation and lipid content of both strains. According to the author, he asserted that 9% carbon dioxide concentration and 17 μmol m^{-2} s^{-1} of light intensity are the optimum. Also, the obtainable lipid content at these conditions is 11.37% for *Nannochloropsis oculata* and 9.50% for *Tetraselmis chuii.*

The drawback here is that, at light intensity of 17 μmol m^{-2} s^{-1} that the author claimed to be the optimal value to achieve a high CO_2 fixation rate by the species studied, however, at outdoor large-scale system, the culturing temperature is expected to be raised higher than at 5 μmol m^{-2} s^{-1}. Therefore, in view of this, the study might not accurately give the optimistic result for outdoor large-scale application.

2.4. Effect of Temperature

The main principal factor that significantly affect the growth of microalgae is the temperature (Kasiri *et al.,* 2015). This affects the rate of growth by directly influencing the rate at which enzyme catalyzed reactions occurs. In general, increment in culturing temperature results to an explosive increment in the microalgae growth since the rate of metabolic activities would the enhanced. However, a drastic reduction in the growth would occur if temperature continue to arise above it tolerance level (Singh and Dhar, 2011). Also, at higher temperature, the solubility of gasses decrease in water (Stepan *et al.,* 2002). Moreover, as said earlier, the optimum value for culturing temperature changes from one microalgae strain to another and is influenced by other factors discussed or yet to be discussed, I.e. light intensity.

However, (Kumar *et al.,* 2010) claimed that from literature, temperature ranging from 77^0F to 95^0F is the best for most microalgae strain while some few strains shows higher adaptability at temperature range of 59^0F to 78.8^0F.

An investigation on the effect of CO_2 concentration varying from ambient (0.0036%) to 20% and culturing temperature varying from 30-50^0C on the growth rate of Chlorella vulgaris have been studied by (Chinnasamy *et al.,* 2009) and established that 6% CO_2 concentration and culturing temeperature of 30^0C gave the highest chlorophyll and biomass concentration of 11 μg mL^{-1} and 210 μg mL^{-1} respectively. Meanwhile the author explicated that no growth was observed at 50^0C. Another study by (Converti *et al.,* 2009) shows that an increment in the culturing temperature from 20-25^0C of two microagae species resulted in double increase in lipid content obtained i.e., for *Nannochloropsis oculata* (88% increase was observed) and a further increase in culturing

temperature from 25–30^{0}C resulted in drastic reduction in lipid content for *Chlorella vulgaris* I.e.(about 59% decrease).

The drawback here is that the author failed to realized that at harsh higher temeperature, some microalgae strain might still show good adaptability at richer nutrient ratios or much higher CO_2 concentration which has not been reported in earlier studies. Therefore, more investigations are needed in this area to comprehend what other parameters or factor could be altered at higher temperature seasons (i.e. summer period) in order to sustain their production at large scale cultivation for biofuel industries.

2.5. Effect of Some Nutrients

2.5.1. Significance of Nitrogen

Nitrogen which is available inform of nitrate represent on of the most crucial macroelements needed for microalgae growth. The element is a constituent of the materials which are directly linked with the principal metabolism of these algae. Alteration in the availabilty of nitrogen results to reduction in the generation of carbohydrates and amino acids. It deficiency has been linked to drastic reduction in fixation of carbon because of low production of bio-catalyst which aids e^- transfer photosynthetically in the chloroplast of a vascular plant as it causes chlorophyll a pigment and hence enhances increment carotenoids (Kumar *et al.*, 2010). Moreover, as reiterated earlier, nitrate among others i.e. urea and ammonium or compounding them remain the most frequently source of nitrogen used.

Prolonging the exponential growth phase is necessary in order to obtain a higher carbon dioxide biofixation rate and specific growth rate (Jin *et al.*, 2006). A study conducted by (Jin *et al.*, 2006)

to investigate the effect of nitrogen on CO_2 fixation rate of microalgae by intermittently controlling the availability of nitrogen in a batch photo bioreactor. The author inferred that supplying of nitrogen intermittently would be a better way achieve higher CO_2 fixation rate in a photo bioreactor. In another study by (Choul-gyun, 2002) , *Chlorella kessleri* was investigated for its efficiency in the removal of nitrogen in treatment of wastewater with small nitrogen to carbon ration (N:C) and the response obtained indicate that, the strain could use up the surplus nitrogen and uses CO_2 bubbled in the media through HCO_3^- ions. Therefore, concluded that the removal would continue so far CO_2 mass transfer is not hindered.

In another study carried out by (Converti *et al.,* 2009), the authur ascertained that in an attempt to optimize the specific growth rate by reducing the concentration of nitrogen by 75% resulted in an increment in lipid content of both species i.e. *Nannochloropsis oculata* and *Chlorella Vulgaris* by 93.7% and 178% respectively.

2.5.2. Significance of Phosphorus

Phosphorus, which is available to microalgae cells in form of phosphate, represents another most crucial macroelement needed for their growth and intrinsic metabolism (Kumar *et al.*, 2010). Availability of phosphorus has been directly linked to the generation of energy required for carbon transport by producing ATP. Also, linked to the utilization of carbon for synthesizing protein through the process of phosphorylation and thereby affecting microalgae's ability to influence CCMs (carbon dioxide concentration mechanisms) (Xu *et al.*, 2010). However, one significant report which solely studied the effect of phosphorus together with different CO2 concentration (Xu *et al.*, 2010). In the report, it is explicated that excess availability of phosphorus enhances microalgae growth due to increasing in the rate of photosynthesis process. A Red alga (*Gracilaria lemaneiformis*) used in the study showed it ability to be

photosynthetically enhanced (by 0.072%) at higher CO_2 concentration and phosphorus level. But the study fails to view the response at higher temperature.

However, two different studies (Cheng *et al.*,, 2010; Yeh & Chang, 2012) also reported on enhancing lipid accumulation of chlorella vulgaris by varying conditions of cultivation which include light intensity, CO_2 concentartion, and nutrient concentration. The results shows a great influenced of these factors and therefore need for optimization arise.

2.6. Methods of Optimization

Method employed in the literature can be broadly categorize into two which are:

1. The mathematical method
2. The statistical method

2.6.1. The Mathematical Method

Many investigations have been carried out to mathematically model microalgae systems where it has been regarded that rate of growth of microalgae is a function of nutrient ratios concentration by Monod equation. In addition, a model known as Michealis – Menten model, has represented the kinetic of nutrient uptake rate. Many other studies proposes several equations by modifying Monod equation in an attempt to incorporate the effect of light intensity (Kasiri *et al.*, 2015).

2.6.2. Statistical Method

Designing of experiment by statistical methods are generally categorize as part of RSM (respond surface method). However, little studies have been reported on optimal experimental design for optimization of microalgae growth rate and CO_2 biofixation rate by microalgae (Ho *et al.*, 2012), (Yewalkar *et al.*, 2011; Zheng *et al.*, 2011). The most common statistical methods on optimal

experimental designs are categorized as part of response surface methodology. Desirability function and response surface metholodogy have be used by (Zheng *et al.,* 2011) to optimize the CO_2 biofixation rate together with starch content of a microalgae *Tetraselmis Subcordiformis* cultivated in a rectangular shaped photo bioreactor by changing the initial concentration of biomass, CO_2 concentration and gas flow rate. Also, (Ho *et al.,* 2012) employed the statistical tool, response surface methodology to optimize CO_2 biofixation rate and specific growth rate by changing the culturing conditions of a strain *Scenedesmus obliquus.* Factors which serves as the input factors for the RSM includes light intensity, carbon dioxide concentration, Mg concentration and gas flow rate. The optimization of $NaNO_3$, PO_4^{3-}, Fe-EDTA and micronutrients concentration for *Chlorella pyrenoidosa* has also been studied by (Yewalkar *et al.,* 2011) grown in 95% oil sand process- affected water media. (Béchet *et al.,* 2013) reported a review on various models that have been developed for studying the effects of temperature and light intensitiy on microalga biomass productivity in an outdoor cultivation.

Several studies concerning the biofixation of CO_2 by microalgae have only been riveted on growth rate of biomass cell and its productivity since there are correlations between CO_2 biofixation rate and the parameters (Zheng *et al.,* 2011; Ho *et al.,* 2012; Anjos *et al.,* 2013; Cheng *et al.,* 2013;). Also, some few studies have been reported on directly evaluating of CO_2 uptake rate from the media (Purba and Taharuddin, 2010) and optimal experimental design of microalgae strains nurtured in oil sand process affected water to optimize the rate of CO_2 uptake rate which measurement is based on directly measuring Carbon dioxide level and concurrently optimizing the specific growth rate and biofixation rate of CO_2 by *chlorella Kessleri* (Kasiri *et al.,* 2015a). Again, the same author but in another study uses nonlinear dynamic model to estimate the optimal

level of carbon dioxide concentration, light intensity and phosphate level concentration to achieve maximum growth rate and CO_2 biofixation rate *chlorella kessleri* (Kasiri *et al.,* 2015).

However, the adeptness to optimizely model microalgae productivity and CO_2 biofixation especially under the varying conditions of temperature, nutrient availability and CO_2 concentration is crucial for evaluating the profitability and sustainability of their cultivation at large scale for biofuel industries and to the best of our knowlegde, no investigation has been done on the optimal experimental design to optimize microalgae specific growth rate, CO_2 biofixation rate and efficiency of wastwater trratment by directly varying culture temperature, nutrient ratios and CO_2 concentration simulteanously.

Therefore, this study aimed to first investigate the effect of CO_2 concentration on the growth of *Chlorella vulgaris, Chlorella kessleri, Chlorella prototheocoides and Neo oleoabundans*) taking into account: (i) specific growth rate; (ii) biomass productivities; (iii) CO_2 fixation rate; and (iv) nitrogen and phosphorus uptake.

Secondly, a central composite response surface design was then employed to develop quadratic regression models for the specific growth rate, CO_2 biofixation rate and rate of nutrient removal by altering the initial nutrient concentration (i.e. nitrate and phosphate), culturing temperature and CO_2 concentration of *Chlorella vulgaris* cultivated in BBM media (batch process). Multiobjective optimization method was employed to determine the maximum specific growth rate, CO_2 biofixation rate and biomass productivity concurrently. These factors were choosen since it have been reported that they have essential implication and effects on the above mentioned outputs (i.e. microalgae specific growth rate, CO_2 fixation rate and it productivity (De Morais & Costa, 2007; Yewalkar *et al.,* 2011; Ho *et al.,* 2012; Razzak *et al.,* 2013).

CHAPTER 3

THESIS OBJECTIVES

3.1 Overall Objective

The primary objective of the study is to investigate the effect of nutrient ratios (N:P), culturing temperature and carbon dioxide concentration on the growth of microalgae biomass, CO_2 fixation and efficiency of wastewater treatment.

3.2. Specific Objectives.

1. Individually and simultaneously study the effect of CO_2 (i.e. 0 - 12%), culturing temperature (i.e. 25 - 60^0C) and nutrient ratios (i.e. P from 1:1 – 8:1) on microalgae growth rate, CO_2 fixation and efficiency of wastewater treatment.
2. Maximize CO_2 uptake rate, biomass productivity and efficiency of wastewater treatment.
3. To investigate the optimal levels of culturing temperature, nutrient ratio and CO_2 concentration by multi-objective optimization.

CHAPTER 4

MATERIALS AND METHODS

4.1. Microalgae Strains and Culturing Conditions

In this study, *Chlorella vulgaris, Chlorella kessleri, Chlorella prototheocoides and Neo oleoabundans* were investigated for their potential use as a CO_2 bio-fixation agent, biofuel production, and wastewater treating agent. The species were obtained from Algae depot in USA and culture in modified Bold's Basal Medium (MBBM).

4.2. Media Composition

Modified Bold's Basal Medium (MBBM) was used for their cultivation. The media consist of $NaNO_3$, $CaCl_2.2H_20$, $MgSO_4.7H_20$, K_2HPO_4, KH_2PO_4, NaCl, EDTA, KOH, $FeSO_4.7H_2O$, H_2SO_4, H_3BO_3, $ZnSO_4.7H_2O$, $MnCl_2.4H_2O$, $CuSO_4.5H_2O$, MoO_3, $Co(NO_3)_2.6H_2O$ dissolved in an appropriate amount of de-ionized water as prescribed by (Andersen, 2013)

4.3. Experimental Procedure and Design

The microalgae species were initially pre-cultured to avoid studying the lag phase and were incubated in batch photobioreactors (1L Erlenmeyer flask) filled up to 1000_ml as a working volume. Four different photobioreactors were inoculated with 1_mg/L of the four different strains, thereby setting the initial biomass concentration to 1_mg/L. The photobioreactors were placed on a bench where Grolux fluorescents light are arranged parallel to the water bath that regulates the culturing temperature between 23-25^{0}C. The ambient air was filtered and mixed with CO_2 with

the aid of the mixing device. As it has been established in several studies that mixing or creating a bubbling environment is required to keep the microalgae cells in suspension, to distribute loosely the nutrients and to obtain equally exposure to the same light intensity and this is expected to be achieved through this mixing device.

For the first study, the CO_2 concentration was varied from 0 i.e. ambient concentration to 6% in four steps. While for the second study, the CO_2 concentration was varied from 0 to 12%, culturing temperature from 25 to 60^oC, and nutrient ratio (NP) from 1:1 to 8:1 all in five steps. The set up as shown in Fig. 4-1 was left for a maximum of 10 to15 days depending on when the growth of each strain reaches their stationary phases just before the death phase set in. The photobioreactor was illuminated with four vertically oriented lux bulbs that provide a light intensity of 60~70 µmol m^{-2} s^{-1}. The microalgae biomass was harvested by centrifuging at 9000RPM for 5min and dried by freeze dryer. Some experiments were replicated and were chosen statistically to exhibit the concept of reproducibility.

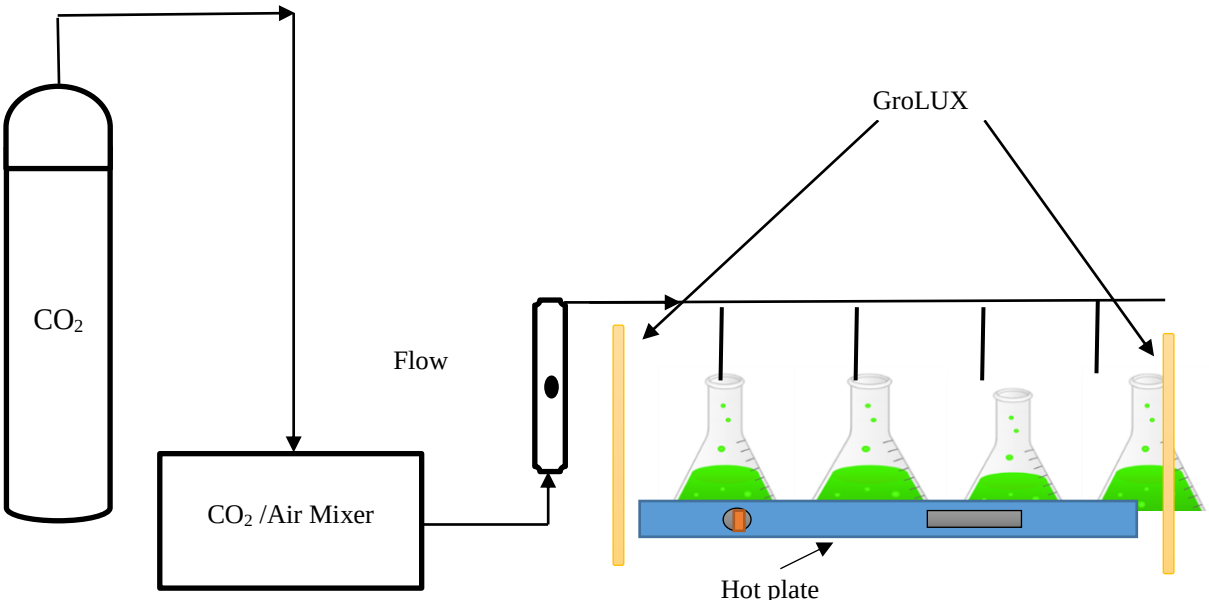


Fig. 4-1: Schematic Diagram of the Experimental Setup

4.3.1. Description of The Analytical Methods

a) **Growth Monitoring Analysis***:* Samples were obtained at 24hrs interval and cell concentration was determine by taken their optical density at 690nm (OD_{690}) using UV-Vis spectrophotometer. Also, the dry biomass weight was obtained by filtering 10ml of the sample and the precipitate was dried in an oven at 50^0C for a period of 24hrs before being weighed.

b) **Growth Kinetic Parameters***:* Parameters like Specific growth rate (μ, d^{-1}), biomass productivity (P, $g_{dw}\ L^{-1}\ d^{-1}$) and CO_2 fixation rate (R_c, $g_{CO2}\ L^{-1}\ d^{-1}$) was obtained by cell concentration and dry biomass weight obtained using the following set of equations respectively.(Feng et al., 2012)

For Specific growth rate, we have:

$$\mu = \frac{lnX_f - lnX_i}{t_f - t_i} \qquad 4\text{-}1$$

Where X_i and X_f represent biomass dry weight at the beginning and at the current day of the exponential growth phase respectively. While t_i and t_f respectively represent the initial and current time (in culturing days) at the same growth phase.

For biomass productivities, we have:

$$P = \frac{X_1 - X_0}{t_1 - t_0} \qquad 4\text{-}2$$

Where X_0 and X_1 represent biomass dry weight at the beginning and at the current day of the exponential growth phase respectively. While t_0 and t_1 respectively represent the initial and current time (in culturing days) at the same growth phase (Feng et al., 2012),(Jacob-Lopes et al., 2009).

c) **CO_2 Measurement:** In order to determine the CO_2 bio-fixation rate R_{CO_2} ($gL^{-1}d^{-1}$), the carbon content analysis is required where the carbon mainly coming from the CO_2 dissolved in the media. CO_2 with different mixing compositions sparging into the media where CO_2 from the gas bubbles transfer to the liquid media. Rate of CO_2 uptake was measured based on the carbon content analysis in the microalgae cell using Eq. 4-3 Rate of CO_2 removal or uptake can also be measured based on concentration of CO_2 measured at the inlet and outlet, but the value cannot be reliable. The only reliable data is the analysis of carbon content in the viable cell based on the productivity relationship. The total carbon content of microalgae was measured using a TOC Analyzer (Teledyne Tekmar® Torch Combustion TOC/TN Analyzer). The carbon content was determined based on the average total organic carbon concentration and the total biomass concentration.

Carbon content values then used to measure rate of CO_2 removal using the following equation:

$$\mathbf{R_C = C_C \,.\, P.} \frac{M_{CO_2}}{M_C} \qquad \textbf{4-3}$$

Where Cc represents the carbon fraction of the produced biomass was determined using TOC analyzer, P represents the biomass productivity and $\frac{M_{CO2}}{M_C}$ represent the ratio of molecular weight of Carbon dioxide to Carbon (Jacob-Lopes et al., 2009).

d) **Nutrients Removal / Wastewater Treatment:** The nutrients removal was estimated by quantifying the level of phosphorus and nitrogen in the culturing medium. The filtrate obtained while performing biomass dry weight was subjected to both nitrate and phosphate test using Chromo tropic acid method and Molybdovanadate method with acid persulfate

digestion respectively according to procedure reiterated in (Hach Company, 2015) and (Hach Company, 2014).

e) **Culturing Temperature***:* A water bath that has the capacity to heat up to 80^0C was used for the experiment.

4.4. Statistical Design of Experiment

Response surface methodology (RSM) is one of a statistical tool employed when the need to determine the correlation between input factors that can be controlled and the response. Moreover, to determine the optimal values of inputs factors that can either minimize or maximize the output response. Generally, according to (Aslan and Cebeci, 2007), RSM uses the form of the following second order model given in Eq. (**4-4**)

$$\boldsymbol{y = \beta + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \beta_{ij} x_i x_j + \sum_{i=1}^{k} \beta_{ii} x_i^2} \qquad 4\text{-}4$$

where x_1, x_2,. …x_k represent the coded values of the independent variables or input factors that determine the output (response) y. In addition, $\boldsymbol{\beta_0, \beta_i, \beta_{ij}}$ and $\boldsymbol{\beta_{ii}}$ represent the unknown parameters that would be obtained by LSR (least squares regression). The steps or levels of the input factors (Z_j), is coded as x_i from Eq. (4-5) provided by (Zheng *et al.*, 2011).

$$x_i = \frac{Z_i - Z_0}{\Delta Z_i}, i = 1,2,3, \ldots\ldots, N \qquad . \qquad 4\text{-}5$$

where, x_i represents the coded value of the true variable, Z_i ; Z_o is the value of Z_i at centre point and ΔZ_i denotes the step change employed in the response surface methodology design.

Two major designs that are commonly used under RSM designs are the central composite and Box-Behnken designs (Toutenburg and Shalabh, 2009). Although, both designs use quadratic

models but the main difference between them is that Box Behnken designs are mostly employed when corner points are either unworkable or insignificant because the experimental points of the input factors are picked at the edge centers of the bounding box. While in central composite design, the experimental points of the input factors are picked on the face centers of the bounding box (Ogunnaike, 2010).

4.4.1. Central Composite Design (CCD)

In the second study, a central composite design as shown on Table **4-1** was employed to generate a set of quadratic regression models for the specific growth rate, CO_2 uptake rate and nutrient uptake rate of *Chlorella vulgaris* and also to consequently determine the optimal levels of culturing temperature, NP ratio and CO_2 concentration. The design was employed to vary the culturing temperature from ($T_L = 25^0C$ and $T_H = 60^0C$), nutrient ratios (N_L =8:1 and N_H = 1:1) and the CO_2 concentration (C_L = 0% and C_H = 12%), since these factors have some considerable effects on the microalgae growth, CO_2 uptake rate specific growth rate and biomass productivity (Razzak *et al.*, 2013). Where L and H denotes extreme high and extreme low levels of those factors respectively. The design included start points which represent extreme values ($-\alpha$, α) as seen on Table 4-1 for each input factors. The coded value of alpha basically depends on the number of factors and as such, $\alpha=2^{3/4} = 1.68$ (Wang, 2006a). The design utilises a matrix of experimental runs according to the number of input factors. Table **4-1** lists the treatment, while **Table 4-2** provides the range and levels of these three independent input factors.

A total number of 20 runs were carried out and randomly sequenced in duplicate to lessen the influence of any kind of experimental error to generate a set of quadratic regression models (Aslan & Cebeci, 2007). Minitab® 17 was employed for completion of regression analysis and Design-Expert® 10 for graphical relationships.

Table 4-1: Central Composite Experimental Design Employed for CO_2, NP Ratio, and Temp.

Experimental Number	CO_2 (x_1)		NP (x_2)		Temperature (x_3)	
	Coded	Actual (%)	Coded	Actual	Coded	Actual (^{0}C)
1	+	10	-	2:1	-	31
2	0	6	-α	1:1	0	39
3	-α	0	0	4:1	0	39
4	0	6	0	4:1	+ α	60
5	-	2	-	2:1	-	31
6	0	6	0	4:1	0	39
7	0	6	0	4:1	0	39
8	+	10	-	1:1	+	50
9	+	10	+	6:1	+	50
10	+	10	+	6:1	-	31
11	-	2	+	6:1	-	31
12	+ α	12	0	4:1	0	39
13	-	2	+	6:1	+	50
14	0	6	+ α	8:1	0	39
15	0	6	0	4:1	0	39
16	0	6	0	4:1	- α	25
17	-	6	-	2:1	+	50
18	0	6	0	4:1	0	39
19	0	6	0	4:1	0	39
20	0	6	0	4:1	0	39

Table 4-2. Configuration of Input Parameters

Parameters	Coded	Actual		
		CO_2 (%)	NP	Temp (^{o}C)
Values range	-α	0	1:1	25
	-	2	2:1	31
	0	6	4:1	39
	+	10	6:1	50
	α	12	8:1	60

4.5. Multi-Objective Optimization Strategy

Multi-objective optimization strategies are employed when optimization involves multiple objectives simultaneously based on trade-offs. In obtaining a single optimal set of solutions, the

objective functions would serve as a sacrificial agent for each other and this was achieved by generating a response optimizer plot using Minitab ® 17. The desirability function (d) or composite desirability function (D) of 1 or value close to 1 is the best.

RESULTS AND DISCUSSION

CHAPTER 5

SINGLE PARAMETRIC STUDY

Herein, we report the effect of single parameters i.e. CO_2 concentration, temperature, and nutrient ratio on the growth responses and CO_2 fixation of microalgae's species.

5.1 Effect of CO_2 Concentration on Microalgae Growth

Just like the terrestrial plants, microalgae also utilize carbon dioxide to carry out photosynthesis in the presence of light. Broadly speaking, raising the carbon dioxide concentration enhances the process of photosynthesis in several microalgae strains due to the fact that, higher availability of CO_2 increases it diffusion from bulk concentration to the active site of Rubisco. But it should be asserted that the increase in CO_2 concentration should not be above the optimum level where the strains can endure (Sato *et al.,* 2003). Microalgae uses HCO_3^- which is formed from the dissolution of CO_2 in the media as shown in Eq. (**5-1**) (Moroney & Somanchi, 1999) below and excess accumulation of this carbonic acid could make the media toxic of which it a degree of effect vary from one strain to another (Razzak *et al.,* 2013). Although, most strain exhibit optimal growth at pH near neutral, however, some prefer acidic environment while others prefer an alkaline medium (Kumar *et al.*, 2010).

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \qquad 5\text{-}1$$

Fig. 5-1 shows the effect of increasing CO_2 concentration on specific growth rates across different strains. For all strains investigated with the exception of *Neo oleoabundans*, an increase in CO_2 concentration above 4% seems to have an adverse effect on the specific growth rate as shown in

Fig. 5-1(a), (b) and (c). This could be best explained due to acidic medium created by dissolving more CO_2 in the media as discussed earlier. However, *Neo oleoabundans* shows some significant increases in specific growth rate above 4% CO_2 concentration till 6% CO_2 concentration as shown in Fig. 5-1(d) which suggest that for the particular strain, probably it can still accommodate higher CO_2 concentration.

This study forms the basis of our future investigation to determine the optimum CO_2 concentration for such strain by employing response surface methodology and perhaps elucidate the regression models to predict the dynamic behavior of microalgae.

The lowest specific growth rate of $0.43d^{-1}$ achieved was with *C. Kessleri* at 6% CO_2 concentration, the light intensity of 36 μmol m^{-2} s^{-1} and initial phosphate concentration of 2Mm which is in a good agreement with (Kasiri *et al.*, 2015b) findings who reported 0.28 d^{-1} at 5% CO_2 concentration. It should be noted that (Kasiri *et al.*, 2015b) uses light intensity of 40 μmol m^{-2} s^{-1} and 29mM of phosphate. Whereas the highest specific growth rate of 1.71_d^{-1} was achieved with C. Prototheocoides at 4% CO_2 concentration as shown in Fig. 5-2. *C. vulgaris* shows a specific growth rate of $1.2d^{-1}$ at 4% CO_2 concentration which is in total agreement with (Gonçalves *et al.*, 2014a) findings who reported $1.2d^{-1}$ for *C. vulgaris* at 4% CO_2 concentration and 24hrs period of light irradiance. Similar specific growth rate values between the microalgae *C. vulgaris* and *P. Subcapitata* were previously reported in the study performed by (Pires and Martins, 2014). However, it might be difficult to compare the various results in the literature, because of the different media, conditions, and strains.

The effect of CO_2 concentration on the growth (note: not growth rate) of all strain investigated is also depicted in Fig. 5-3. As observed, the cell concentration in terms of optical density (OD) at

690nm and dry cell weight was plotted against various CO_2 concentration. The cell dry weight was calculated from the calibrated curves shown in Table **5-1**. As one would expect, the higher the OD_{690} which represent cell concentration, the higher the dry cell weight, but this is not the case. OD_{690}nm only measures the chlorophyll content of the reactor for example in Fig. 5-3(a), despite having such high OD_{690}nm at 6% CO_2 concentration, however, the dry cell weight was found to be 0.52_gL^{-1} even lower than dry weight achieved at 2% CO_2 concentration.

Table 5-1: Calibration Curves of OD_{690}nm and Cell Conc.

Microalgae strain	$\boldsymbol{OD_{690} = mx_{(gdw/L)} + b}$	$\mathbf{R^2}$
Chlorella vulgaris	$3.4638x - 0.0346$	0.999
Chlorella kessleri	$4.4883x - 0.0449$	0.9901
Chlorella prototheocoides	$3.5361x - 0.0354$	0.9917
Neo oleoabundans	$3.1606x - 0.0316$	0.9921

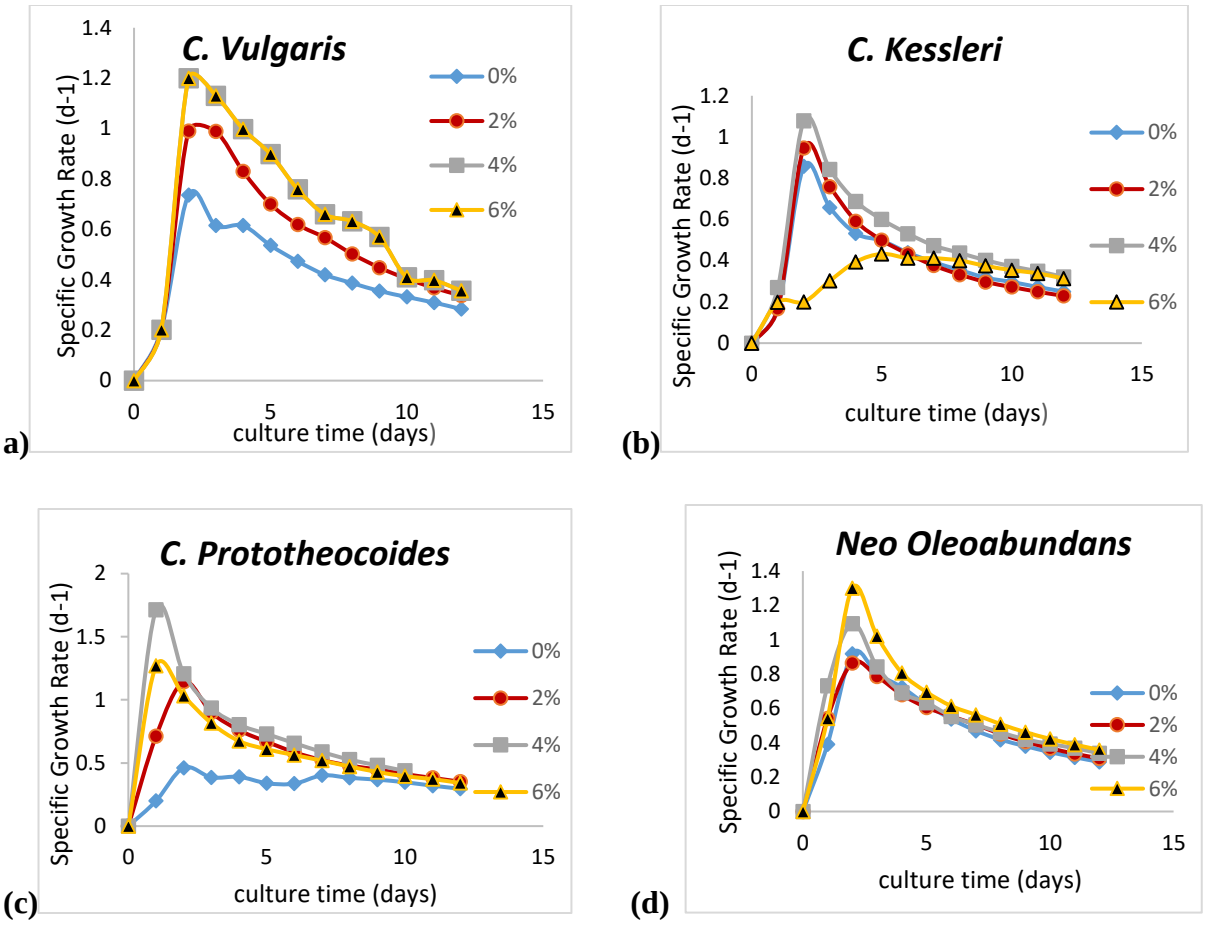


Fig. 5-1: Effect of CO_2 Concentration on SG (a) Cv, (b) Ck, (c) Cp and (d) Neo O

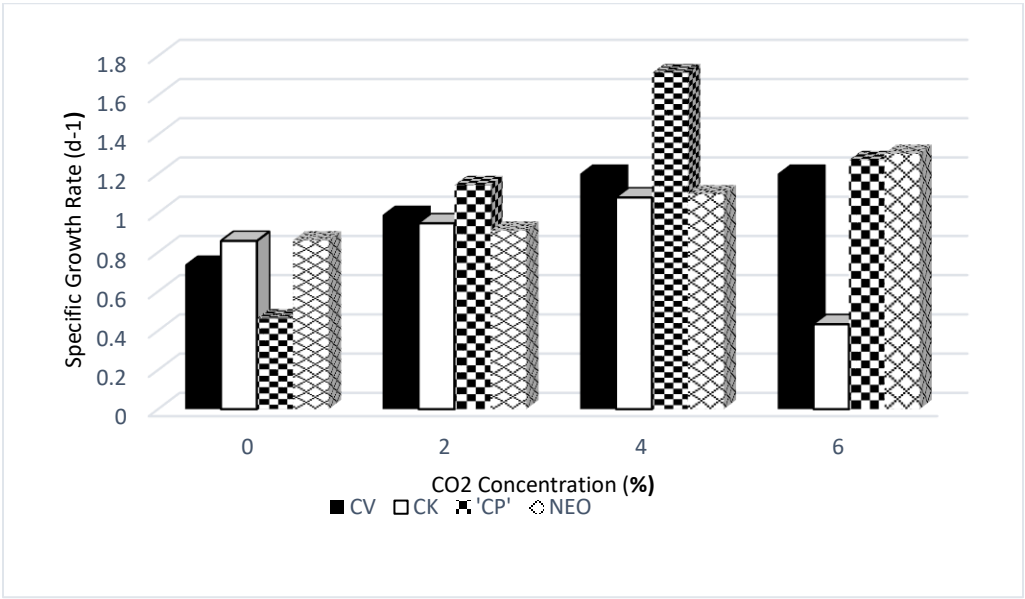


Fig. 5-2: Maximum Specific Growth of all Strains at different CO_2 Concentration

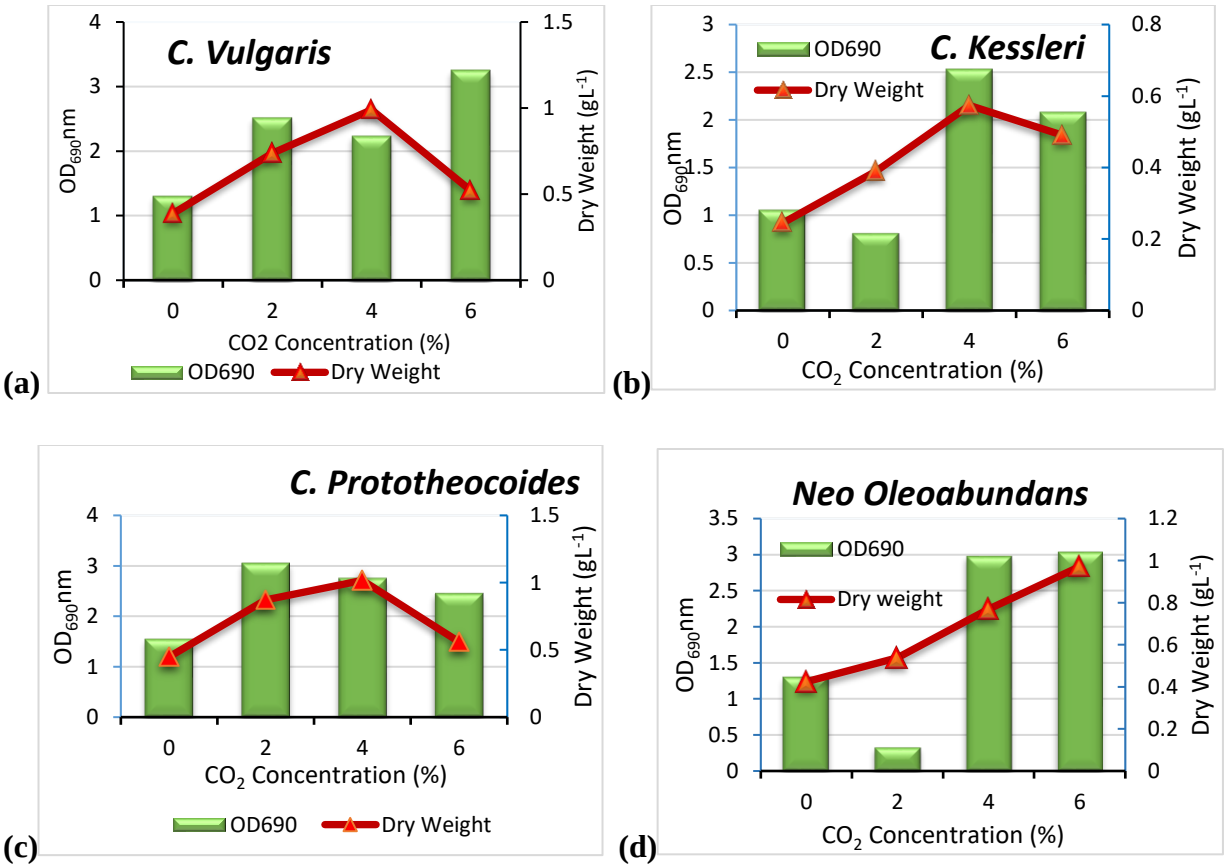


Fig. 5-3: Maximum value of OD_{690}nm and dry weight obtained at different CO_2 conc.

5.2. Single Parametric studies on Biomass productivities and CO_2 fixation rate

Information about the average composition of microalgae biomass, as well as biomass productivities can be used to determine carbon dioxide uptake rate, assuming that all the CO_2 assimilated was converted into biomass and at which temperature is most favored. Fig. 5-4, **Fig. 5-8** shows biomass productivities and CO_2 biofixation rate determined from Eq. (4-2) and Eq. (4-3) respectively at different CO_2 concentrations while Fig. 5-7 shows the effect of temperature and Fig. 5-9 revealed the effect of nutrient ratio on CO_2 fixations rate of *Chlorella vulgaris*.

5.2.1. Biomass Productivities

Regarding biomass productivities, a similar behavior was observed as shown in Fig. 5-4. In general, higher CO_2 concentration led to an increase in maximum biomass productivities. The lowest

biomass productivity, 0.027_g/L/day, was achieved for the *C. kessleri* at 0% CO_2 concentration which in agreement with (Gonçalves *et al.*, 2014b) findings who reported lowest biomass productivity of 0.022_g/L/day for *P. subcapitata.* On the other hand, the highest biomass productivity value, 0.11_g/L/day, was achieved by the *C. prototheocoides* at 6% CO_2 concentration. *C. vulgaris* and *Neo oleoabundans* showed a similar behavior in terms of biomass productivity. The highest values achieved were 0.09_g/L/day at 2% CO_2 concentration and 6% CO_2 concentration respectively. The increase in CO_2 concentration favors maximum biomass concentrations. These results suggest that all the studied strains with the exception of *Neo oleoabundans* behave similarly when the CO_2 concentration is increased. However, the highest productivity values for *Neo oleoabundans* could not be obtained here in the chosen range of our CO_2 concentration, which indicates that higher biomass productivity could still be achieved for this strain when cultivated at higher CO_2 concentrations (say 8-12%).

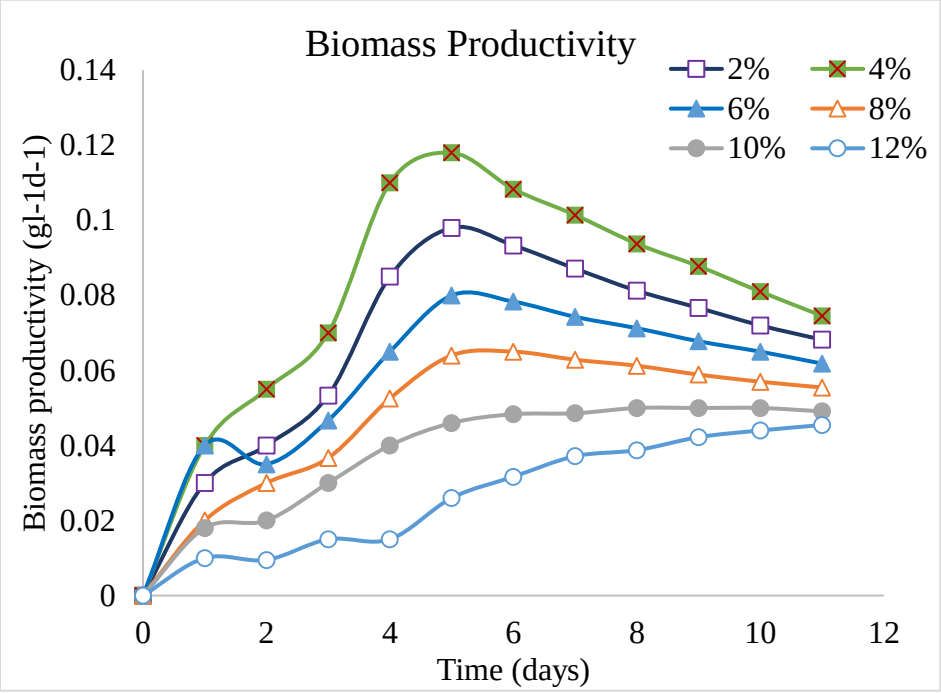


Fig. 5-4: Effect of CO2 concentration on biomass productivity of *Chlorella vulgaris*

5.2.2. CO_2 Biofixation Rate

For all microalgae strains investigated, an increase in CO_2 concentration resulted in an increase in CO_2 biofixation rate as shown in **Fig. 5-8** and Fig. 5-10(b). The increase observed could be best explicated by the luxurious uptake of CO_2 at elevated CO_2 concentrations. We speculated that the microalgae strains seek to hive away surplus nutrients (i.e. carbonate) in its intracellular pools for usage in the period when nutrient starvation set in since a good number of algal strains shows this conduct (Liang *et al.*, 2009). However, at higher CO_2 concentration (say 6%), as show in Fig. 5-5, the rate of CO_2 biofixation reduces and this is expected since the culturing media become acidic as observed in Fig. 5-6 thus put the microalgae in a harsh toxic environmental condition.

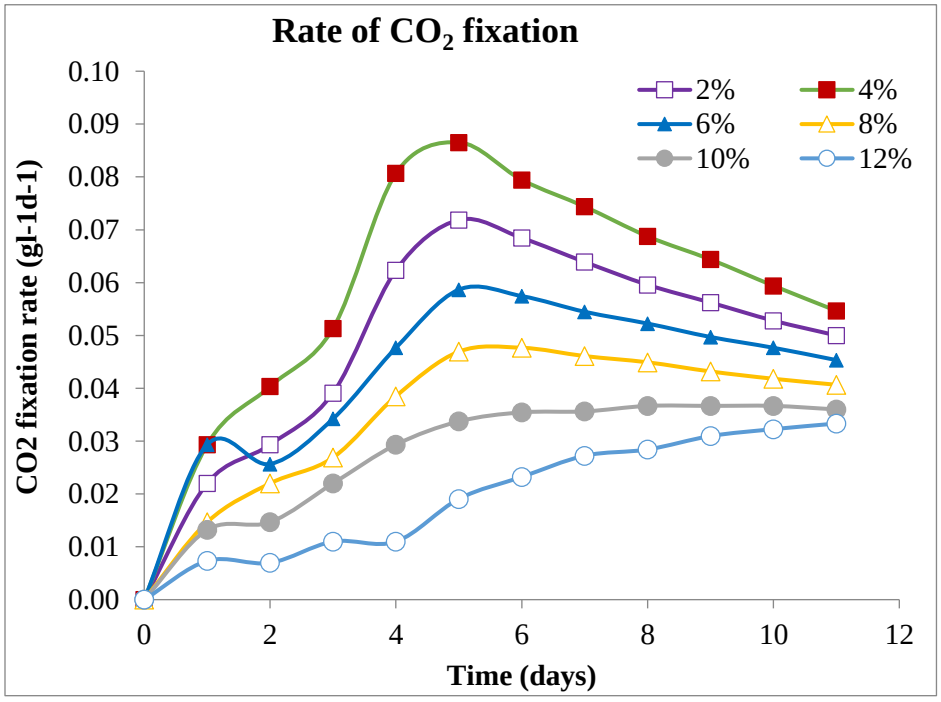


Fig. 5-5: Effect of CO_2 Concentration on CO_2 fixation rate

An increase in biomass productivities and in CO_2 uptake rates with increasing light irradiance has already been described (Cheng *et al.*, 2006; Morais *et al.*, 2007; Chiu *et al.*, 2008; Li-haiFan *et al.*, 2008). In fact, at CO_2 concentration below the optimum value, photorespiration rate is directly

proportionally to CO_2 biofixation rate, resulting in an increase in biomass productivities and in CO_2 uptake. For irradiance values above the light saturation point, an over-dissolution process occurs, damaging the photosystems and inhibiting photosynthesis and microalgae growth (Sobczuk *et al.*, 2000; Yr *et al.*, 2003; Chinnasamy *et al.*, 2009).

These results have shown that microalgae culturing can be effective in CO_2 capture from the atmosphere, which may reduce costs associated with CO_2 supply. All studied microalgae strains seem to be effective in CO_2 capture due to their high biomass productivities, being promising alternatives for large scale production

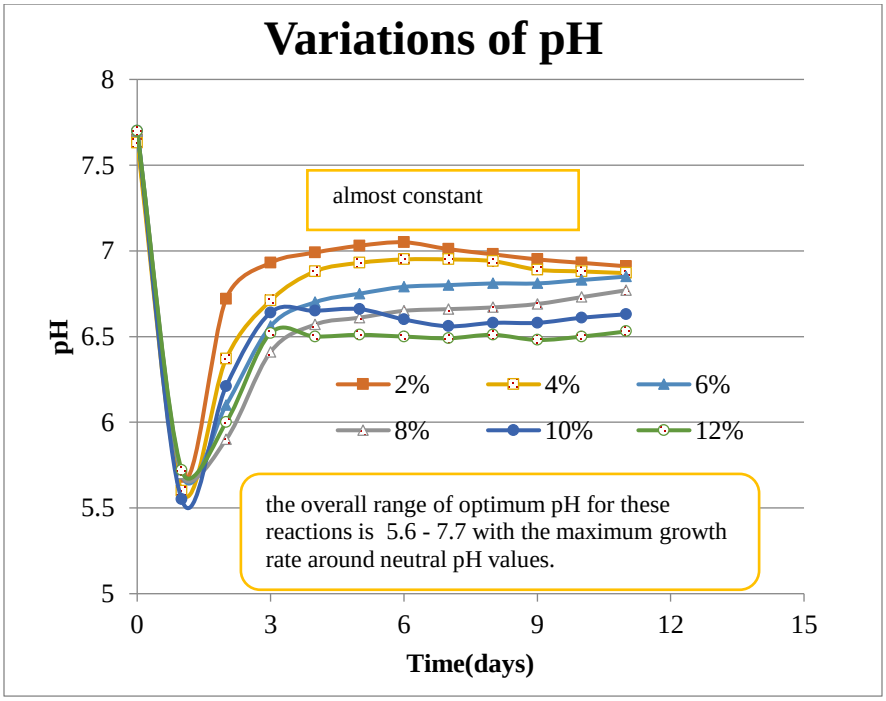


Fig. 5-6: Effect of CO_2 concentration on the media Ph

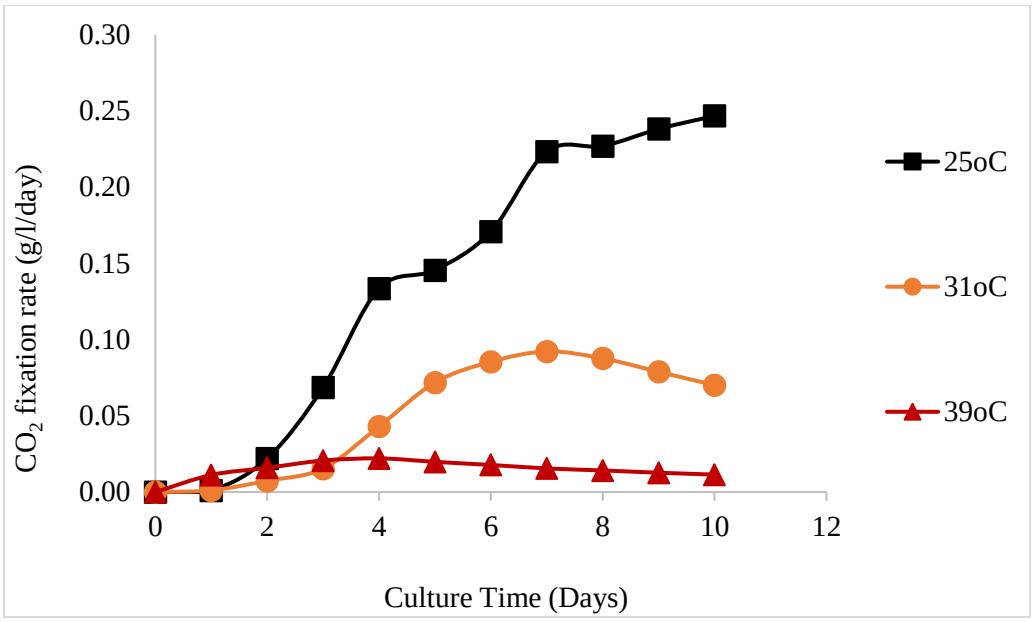


Fig. 5-7: Effect of temperature on CO_2 fixation rate at 4% CO_2 concentration

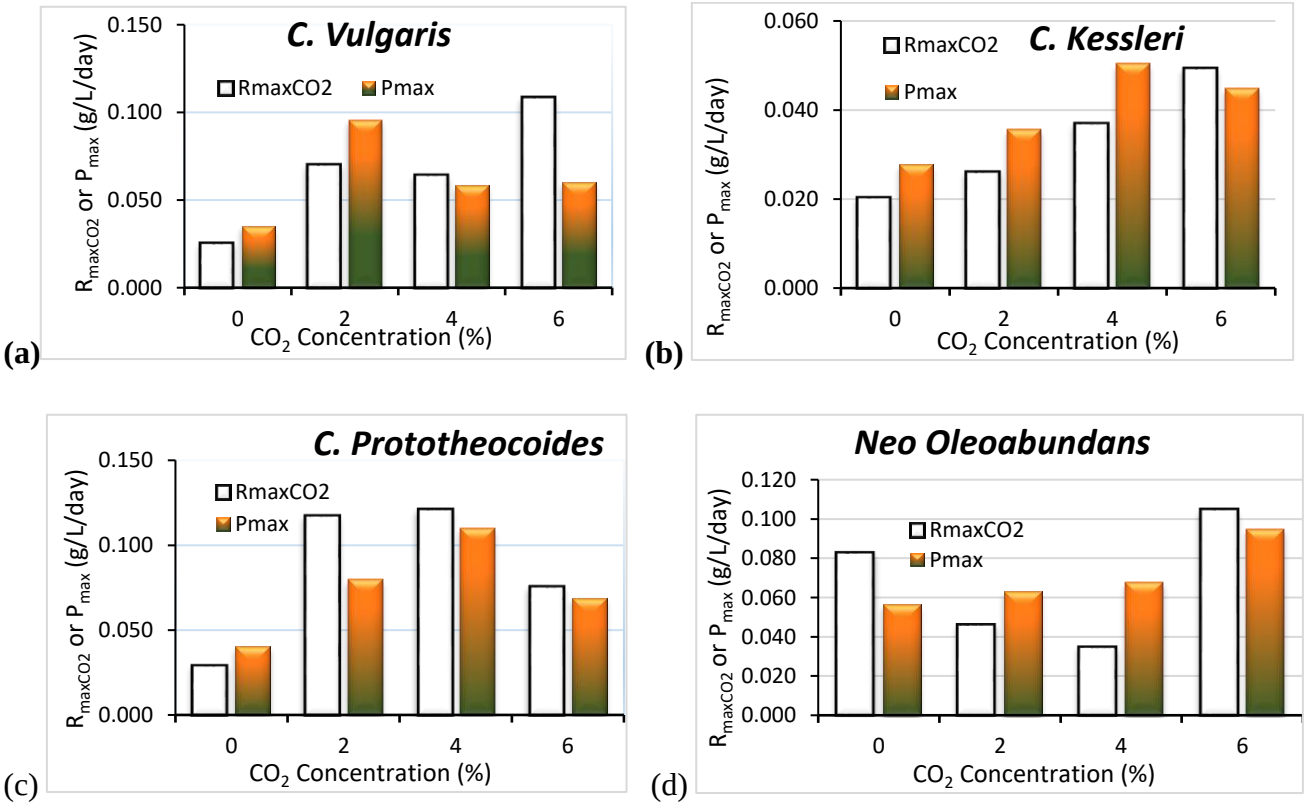


Fig. 5-8: CO_2 fix and BP of all strains at different CO_2 conc.

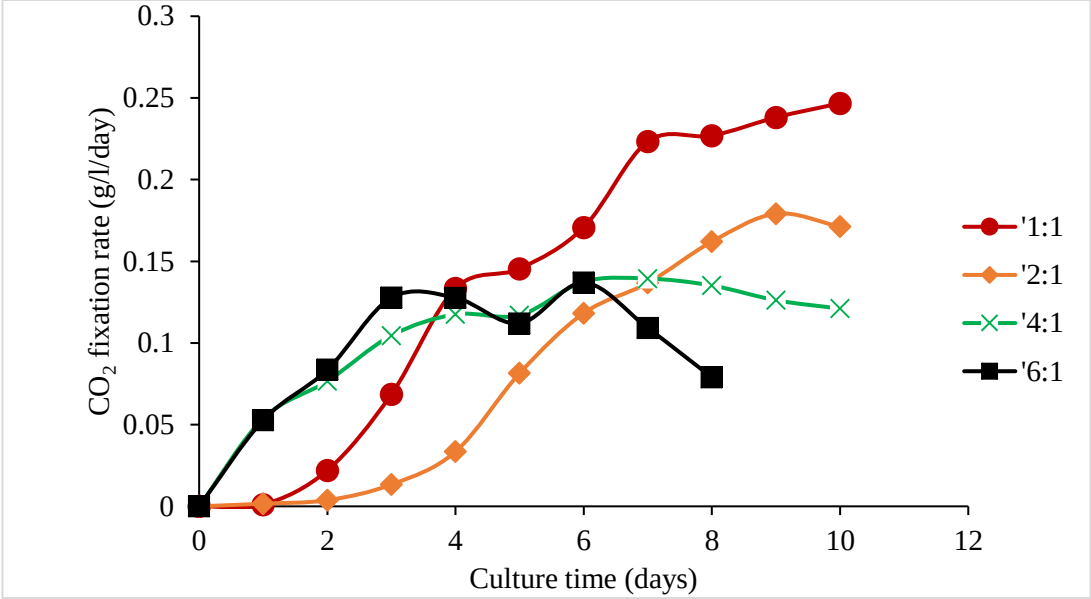


Fig. 5-9: Effect of nutrient ration on CO_2 fixation rate at 4% CO_2 concentration.

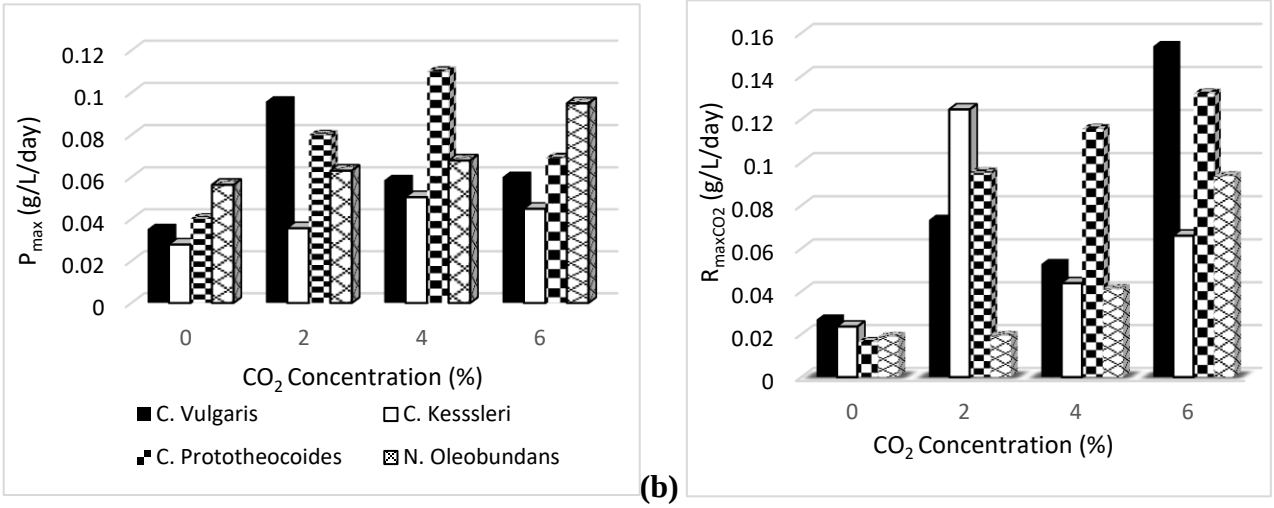


Fig. 5-10: Comparism of (a) BP and (b) CO_2 fix. at different CO_2 concentration

5.3. Effect of CO_2 Concentration on Efficiency of Nutrient Removal

The culture supernatant was analyzed on daily basis throughout the culturing for nitrate and phosphate to determine the efficiency of their removal by the strains as shown in Fig. 5-11.

As expected, the nutrients removal efficiency was poor at the beginning of the culturing days (Day 0–2 or 3) due to low cell concentration (0.01_g/l) as shown in Fig. 5-11 (a) and (b). It is expected that the higher the cell concentration, the better the nutrient removal efficiency (Lau *et al.*, 1995). Thereafter, the removal efficiency of nutrient achieved higher level during the growth phase, due to the higher cell concentration and vigorous growth. It was interesting to note that from Fig. 5-11 (b), the removal of Phosphorus from the media was very low and almost most strains in the literature including those investigated in this study shows this trend. This is because Microalgae do not require Phosphate in such high amount as available in wastewater despite being one the most crucial macroelement needed for their growth and intrinsic metabolism (Kumar *et al.*, 2010).

(a) Concerning nitrogen removal, when the lowest CO_2 concentration was applied (i.e. 0.03%-ambient air), all microalgae strains showed reduction percentages lower than the values established by EU legislation (64%). However, when higher CO_2 concentration (i.e. 4-6%) was applied, percentages of reduction higher than 70% were obtained for all cultures except for the *C. kessleri* which gave 65% reduction at 4% CO_2 concentration as shown in Fig. 5-12 (a). However, at 6% CO_2 concentration, all strains showed a reduction percentage of about 100% as observed from Fig. 5-12 (a).

These results show that higher CO_2 concentration favors nitrogen removal and that, in general, all studied strains can be effectively applied in nitrogen removal. High nitrogen removal percentages have been described in different studies. In the study performed by (Xin *et al.*, 2010), the microalga

scenedesmus sp. was able to remove 90.4% of nitrate after 13 days of cultivation with an initial nitrate concentration of 10_mg L^{-1}, a CO_2 concentration of 2%. A nitrogen removal efficiency of 82.70% was obtained for the microalga *Chlorella zofingiensis* at 2% CO_2 concentration (Zhu *et al.*, 2013).

(b) Regarding phosphorus uptake, removal efficiencies were far from satisfactory, as the minimum percentage of reduction established by EU legislation, 80%, was not achieved lower CO_2 concentration. However, it is possible to state that increasing CO_2 concentration results in higher phosphorus removal. In this study, all strains investigated showed a similar behavior in terms of phosphorus uptake as shown in Fig. 5-12 (b). However, the highest phosphorus removal, about 88.85%, was achieved by the *C. kessleri* at 6% CO_2 concentration. Phosphorus removal efficiencies obtained in this study were in agreement with those referred in the literature. Phosphorus removal percentages close to 100% were obtained for the microalgae *scenedesmus sp.* and *C. zofingiensis* in the studies performed by (Xin *et al.*, 2010; Zhu *et al.*, 2013) respectively.

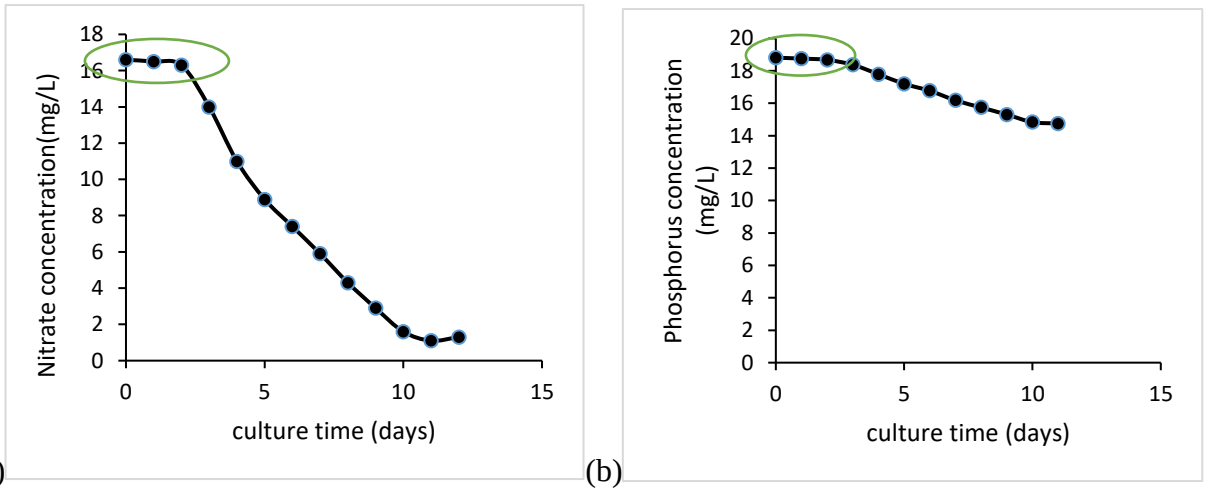


Fig. 5-11: Removal of Nutrients in the media by a typical Microalgae (a) NO_3 removal (b) P removal

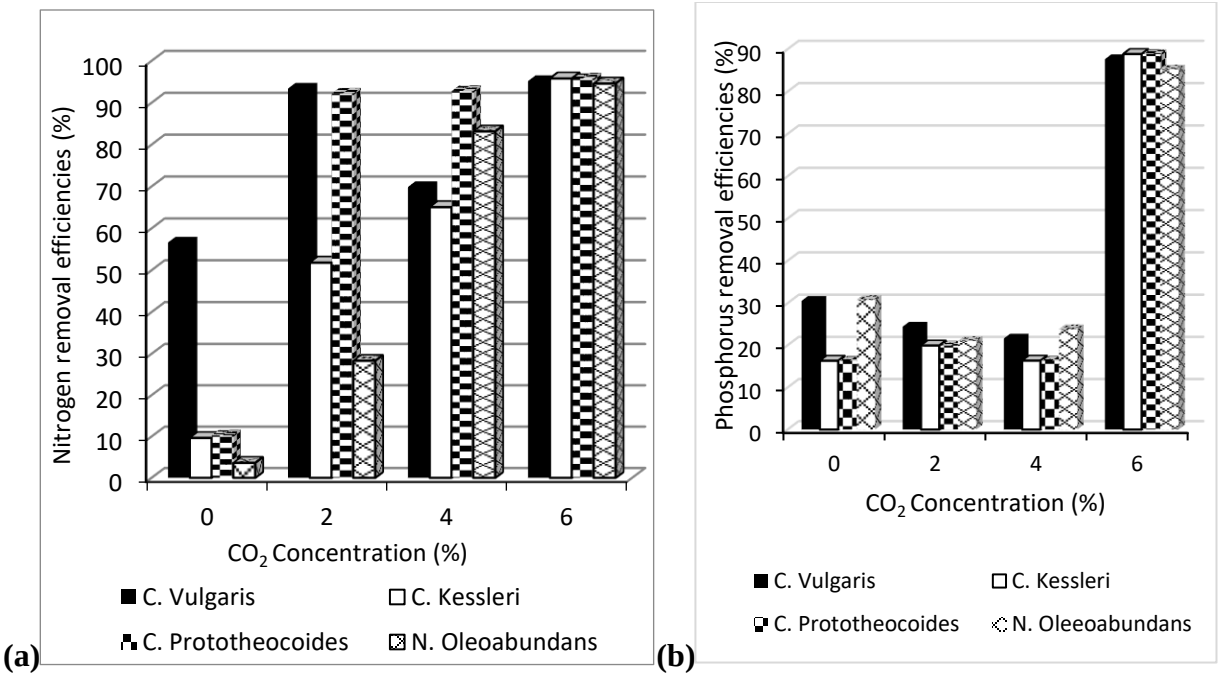


Fig. 5-12: % removal of Nutrient (a) N_2 removal and (b) P removal

CHAPTER 6

OPTIMIZATION OF CO_2 FIXATION RATE AND BIOMASS PRODUCTIVITY.

Microalgae cultivation and their use is a promising approach for integrated CO_2 biofixation, wastewater treatment and renewable energy production. To develop such an important technology, there is a need to optimize the culture conditions, maximizing CO_2 consumption, degrading the nutrients present in the wastewater and maximise the microalgae biomass production.

the optimization of three parameters in just five steps while employing classical n-factorial approach would require5^3 or 125 individual runs. To avoid such large number of runs, a statistical design of experiment (i.e. DOE) could be employed. This approach includes central composite design and Box-Behnken design. It also enables the identification of synergistic relationships between input parameters. The present study considered the use of statistical central composite design technique to model specific growth rate, biomass productivity and CO_2 biofixation rate of *Chlorella vulgaris* cultivated in a modified bold's basal medium by adjusting different nitrogen-to-phosphorus ratio under different culture conditions (i.e. temperatures, nitrogen-to-phosphorus ratio, CO_2 concentration). The optimal levels of nutrient ratio, the percentage of CO_2 and culturing temperature were determined by developing a quadratic model for specific growth rate, biomass productivity and the rate of CO_2 biofixation.

6.1. Development of Model

Experimental data obtained from central composite design (CCD) presented in **Table 6-1** are used in the development of the following quadratic regression equations to adequately depict specific growth rate, CO_2 fixation rate and biomass productivity of *Chlorella vulgaris*.

$$\text{Specific growth rate, } \mu = 1.877 + 0.009x_1 - 0.343x_3 + 0.064x_2 - 0.305x_1^2 - 0.682x_3^2 - 0.148x_2^2 - 0.022x_1x_3 + 0.003x_1x_2 - 0.081x_2x_3 \quad (6\text{-}1)$$

$$CO_2 \text{ Fixation Rate } R_{CO2} \text{ (g/l/day)} = 0.02325 - 0.00418\,x_1 - 0.05331\,x_3 - 0.00400\,x_2 - 0.00608\,x_1^2 + 0.01901\,x_3^2 + 0.02257\,x_2^2 + 0.00630\,x_1x_3 - 0.00282\,x_1x_2 + 0.00371\,x_2x_3 \quad (6\text{-}2)$$

$$\text{Productivity } P = 0.05910 - 0.00123\,x_1 - 0.02803\,x_3 + 0.00020\,x_2 - 0.01270\,x_1^2 - 0.01055\,x_3^2 + 0.00277\,x_2^2 + 0.00180\,x_1x_3 - 0.00232\,x_1x_2 + 0.00022\,x_2x_3 \quad (6\text{-}3)$$

where, x_1, x_2 and x_3 denotes the coded values of the CO_2 concentration, NP ratio and culturing temperature respectively.

Fig. 6-1 a-c shows the plots of experimental data versus model predictions for specific growth rate, CO_2 biofixation rate, and biomass productivity, respectively. The coefficient of determination (i.e. R^2) of the quadratic regression models for the specific growth rate, CO_2 fixation rate and productivity were 0.80, 0.98 and 0.91, respectively showing that the regression models (except specific growth rate) developed can effectively depict the relationship between the investigated factors and responses.

Table 6-1: Specific Growth Rate, CO_2 Fixation Rate and Productivity Obtained from the CCD

Experimental number	Specific growth rate (d^{-1})	CO_2 fixation rate ($mgL^{-1}d^{-1}$)	Biomass Productivity ($mgL^{-1}d^{-1}$)
1	0.70	117.33	63.54
2	1.86	94.91	76.36
3	1.50	8.46	34.64
4	0.00	0.00	0.00
5	0.63	131.27	61.47
6	1.88	22.12	55.72
7	1.90	23.00	56.00
8	0.00	0.00	0.00
9	0.00	0.00	0.00
10	1.04	91.20	53.36
11	0.94	127.69	69.89
12	1.46	4.49	33.20
13	0.00	0.00	0.00
14	1.99	80.12	79.03
15	1.63	25.00	55.50
16	0.82	154.91	80.04
17	0.00	0.00	0.00
18	1.93	25.00	66.00
19	1.88	22.12	62.00
20	1.88	22.12	55.72

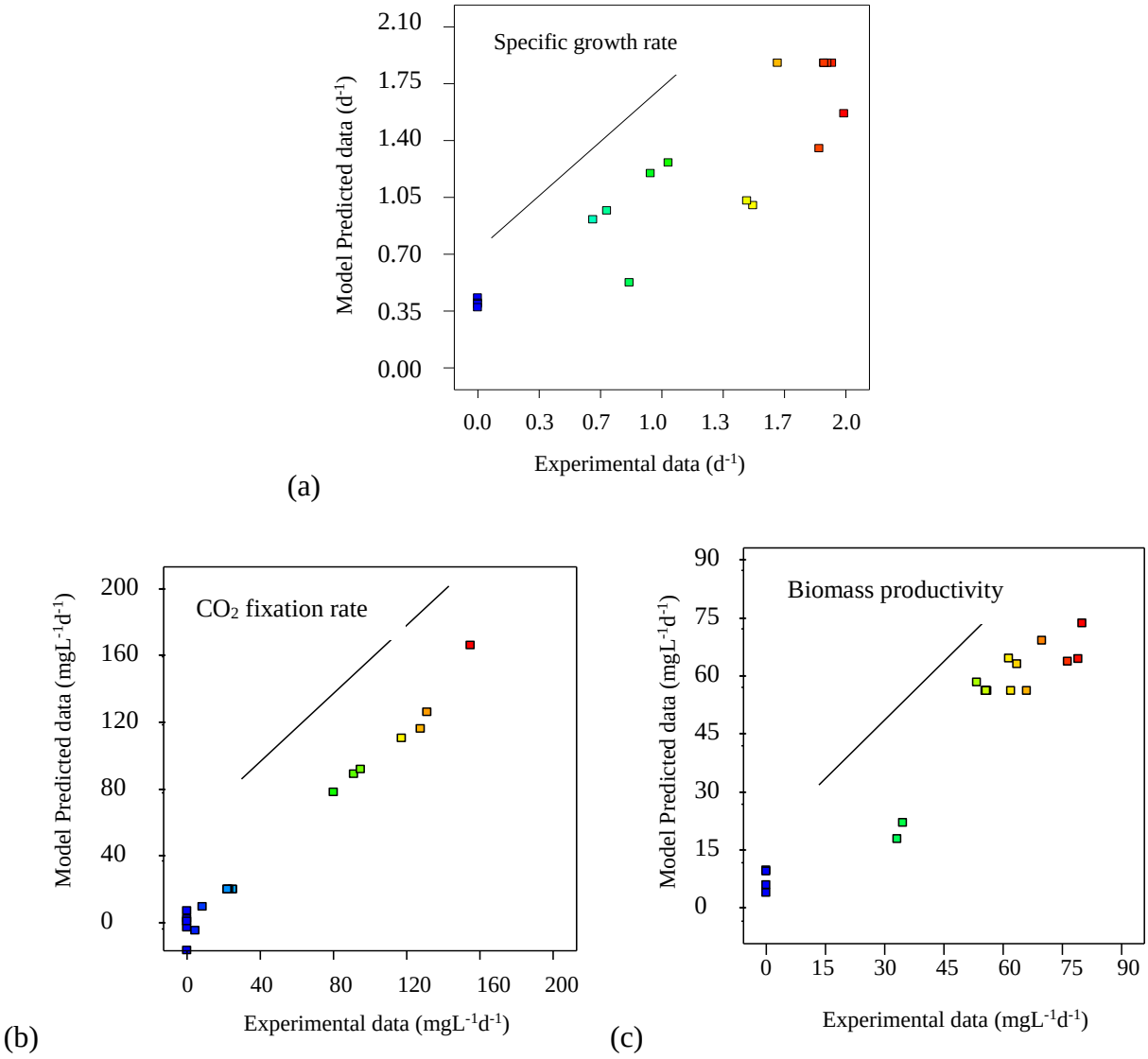


Fig. 6-1: Model Validation (a) SG (R^2 = 0.80), (b) CO_2 fix. (R^2 = 0.98) and (c) BP (R^2= 0.91).

Test of significance for the quadratic regression models of specific growth rate, CO_2 fixation rate, and productivity was carried out by employing analysis of variance (ANOVA) (Ogunnaike, 2010), which is shown in Table 6-2. Here, the analysis is considered to be significant when the p-value in the model term is less than (<) 0.05. As shown in Table 6-2a, the culturing temperature (x_3, p = 0.025) and CO_2 concentration (x_1^2, p = 0.037) are significant factors on the response, specific

growth rate. Table 6-2b shows that the culturing temperature ($x_3, p = 0.00$) and CO_2 concentration ($x_1^2, p = 0.003$) have significant effect on the biomass productivity while NP ratio has no or little significance in the investigated range. As shown on Table 6-2c, when taking CO_2 biofixation rate as the response, the culturing temperature (x_3, p = 0.00), CO_2 concentration (x_1^2, p = 0.018) and NP ratio (x_2^2, p = 0.000) show significant effects. Moreover, the interaction term between the initial CO_2 concentration and culturing temperature ($x_1^2 x_3^2$, p= 0.055) shows little significant effect on the CO_2 biofixation rate.

Table 6-2. Analysis of Variance (ANOVA) for (A) SG, (B) BP, and (C) CO_2 Fix

Term	Coefficient (β)	Standard error	t-Value	p-Value
Constant	1.88	0.20	9.54	0 ς
x_1	0.01	0.13	0.07	0.95
x_3	-0.34	0.13	-2.63	0.03 ς
x_2	0.06	0.13	0.49	0.64
x_1^2	-0.31	0.13	-2.4	0.04 ς
x_3^2	-0.68	0.13	-5.37	0 ς
x_2^2	-0.15	0.13	-1.16	0.27
x_1x_3	-0.02	0.17	-0.13	0.90
x_1x_2	0.00	0.17	0.02	0.99
x_2x_3	-0.08	0.17	-0.47	0.65

(a) Specific growth rate

Term	Coefficient (β)	Standard error	t-Value	p-Value
Constant	0.06	0.005	11.90	0.000ς
x_1	-0.00	0.003	-0.37	0.72
x_3	-0.03	0.003	-8.51	0.000ς
x_2	0.00	0.003	0.06	0.95
x_1^2	-0.01	0.003	-3.96	0.00ς
x_3^2	-0.01	0.003	-3.29	0.01ς
x_2^2	0.00	0.003	0.86	0.41
x_1x_3	0.00	0.004	0.42	0.68
x_1x_2	-0.00	0.004	-0.54	0.60
x_2x_3	0.00	0.004	0.05	0.96

(b) Biomass productivity

Term	Coefficient (β)	Standard error	t-Value	p-Value
Constant	0.02	0.003	6.95	0 ς
x_1	-0.00	0.002	-1.89	0.09
x_3	-0.05	0.002	-24.03	0.00 ς
x_2	0.00	0.002	-1.80	0.10
x_1^2	0.01	0.002	-2.82	0.02 ς
x_3^2	0.02	0.002	8.81	0 ς
x_2^2	0.02	0.002	10.45	0.00 ς
x_1x_3	0.01	0.003	2.18	0.05 ς
x_1x_2	0.00	0.003	-0.97	0.35
x_2x_3	0.001	0.003	1.28	0.23

(c) CO_2 fixation rate

ς Significant at p-value < 0.05

6.2. Effect of Culture Variables

In order to achieve better apprehension of the results, quadratic regression models Eq. (6-1), (6-2) and (6-3) developed were employed to generate three – dimensional (3D) response surfaces and two – dimensional (2D) contour plots, which are presented in Fig. 6-2, Fig. 6-4 and **Fig. 6-5**. The synergetic effects with the several combinations of input factors on specific growth rate, CO_2 biofixation rate and biomass productivity were observed. It is important to note that both surface and contour plots were plotted for 2 factors and the remaining factor was fix at a center level.

6.2.1. Synergetic Effects of Input Parameters on Specific Growth Rate

The understanding of the synergetic effects of temperature, CO_2 concentration and NP ratio on specific growth rate is very important for efficient process operation. Generally, temperature efficaciously controls many metabolic activities (Mun & Guieysse, 2006). Also, stress tolerance ability of microalgae differs from one strain to another. The synergetic effects of culture temperature and CO_2 concentration on specific growth rate are shown in both 3D response surface Fig. 6-2 (a) and 2D contour plot Fig. 6-2 (b). It is obvious that the specific growth rate increases with the increase of initial CO_2 concentration and culture temperature. The maximum specific growth rate was obtained at around 6% CO_2 and 34 ^oC which is in agreement with Chinnasamy *et al*., (2009). The stimulatory effect of CO_2 (up to 6%) on specific growth rate could be related to the increased availability of key enzymes in carbon metabolism such as carbonic anhydrase enzyme and Rubisco, resulting in enhanced photosynthesis (Xia & Gao., 2005). It is interesting to note that both factors significantly affect the specific growth rate which is also in agreement with ANOVA shown in Table 6-2 (a). Nevertheless, the specific growth rate concomitantly decreases

at an elevated temperature above 35^{o}C. The inhibitory effect of CO_2 on specific growth rate was also observed

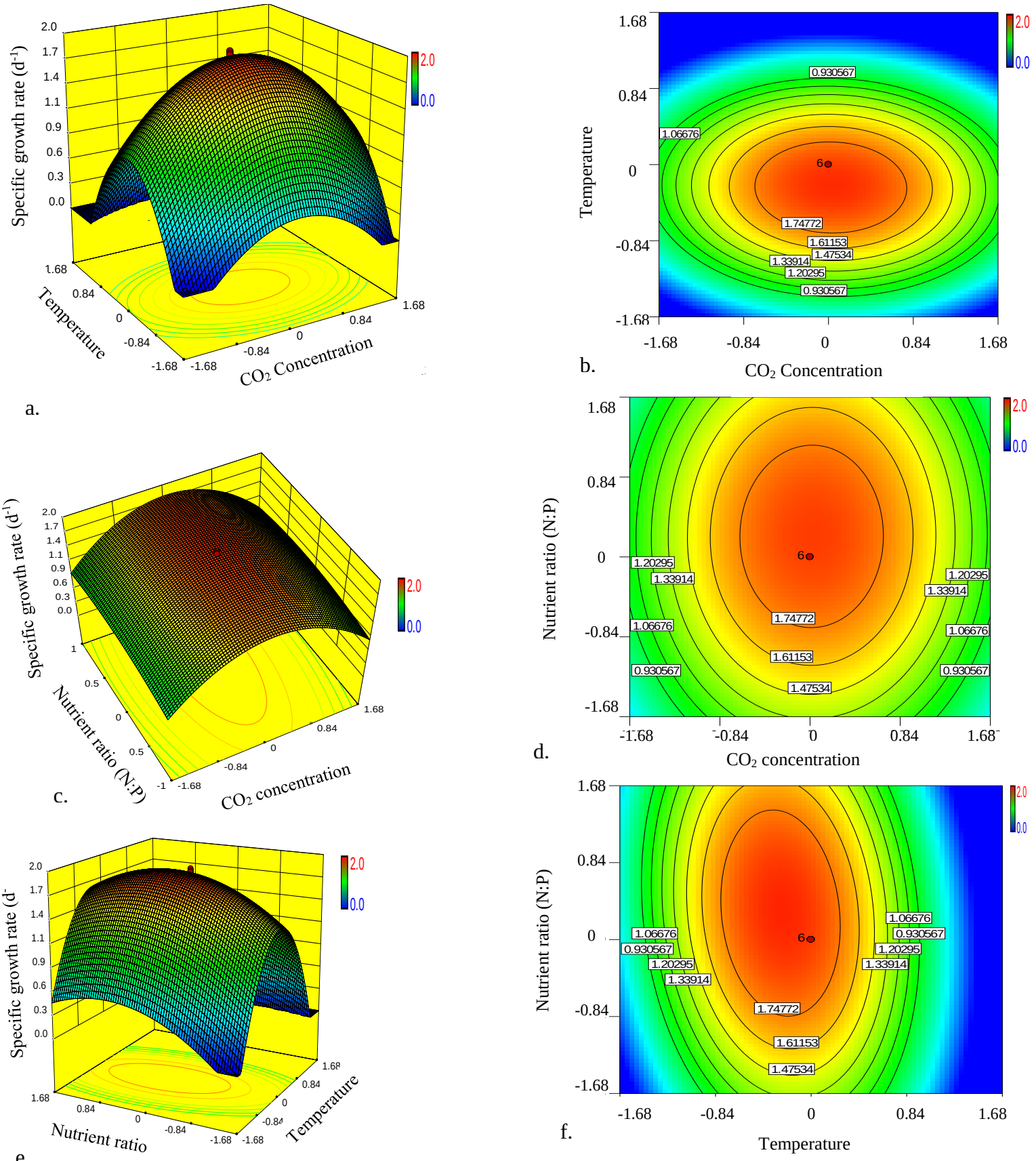


Fig. 6-2: 3D response surfaces and 2D contour lines for the SG

when the level of CO_2 was above 6%. The possible explanation is that the uses of higher CO_2 levels (above 6%) can result in low pH due to carbonic acid formation, which in turn cause the decrease of the activity of the key enzymes (e.g., carbonic extracellular anhydrase and Rubisco) and as such inhibit cell growth (Chen *et al.*, 2011).

Fig. 6-2c and 6.2d show the interaction effects of CO_2 concentration and NP ratio on the specific growth rate. The specific growth rate increases with both CO_2 concentration and NP ratio of 2:1 to 4:1, while it decreases at NP ratio of 1:1, 6:1 and 8:1 respectively. This is probably due to high concentration of phosphate in 2:1 and 4:1 in comparison with those of 6:1 and 8:1. The data indicate that the specific growth rate increases with phosphate concentration but decreases at an extremely high concentration of phosphate showing an adverse effect of phosphate concentration i.e. (1:1). The data are in agreement with the ANOVA test. The results also ratify previous findings of Kasiri *et al.*, (2015).

Fig. 6-2e and 6.2f present the interaction effect of culture temperature and NP ratio on specific growth rate. It reaches its peak at around NP ratio of 4:1 and culture temperature of 34^0C. The strain shows about 10% decrease in its specific growth rate at 35^0C as compared to 33^0C. Further increment in the culture temperature (i.e. 39^0C and above) results in a sudden disruption of the microalgae growth which eventually led to the death of the microalgae cells. This was easily observed from the change of the color from green to brownish suspension. No growth was observed at a culture temperature of 50^0C and above despite altering other culturing conditions. However, this observation is in agreement with Converti *et al.*, (2009) findings.

6.2.2. Synergetic Effect of Input Parameters on CO_2 Biofixation Rate

Temperature is the most influencing factor on microalgae growth and CO_2 fixation rates due to its direct relationship with microalgae metabolic rates i.e. enzymatic reactions (Boyd et al., 2013;

Duarte, 2007). In general, the rate of microalgae growth, CO_2 fixation and metabolic activities increases with temperature till optimal level is attained for a particular species. Temperature increases above optimal value results to decrease cellular metabolic activities since cell death strats at elvated temperure (Raven & Geider, 1988).

Fig. 6-4 **a & b** show the interactive effect of CO_2 concentration and culture temperature on the CO_2 fixation rate. It was observed that the CO_2 fixation rates increases as we decerease the culturing temperature from 60^{o}C to 25^{o}C. However, the CO_2 fixation rates remain zero until the decrease in temperature reaches 39^{o}C. This indicate that the growth of Chlorella vulgaris can not be sustained at culturing temperture above 39^{o}C, no matter what other factors were altered. Originally, the CO_2 fixation rates increases with increase in CO_2 concentration until an optimum concentration level is reached as shown in the single factor experiment on Fig. 6-3. This increase can be explained by the luxury uptake of CO_2 at elevated CO_2 concentrations. The hypothesis is that the microalgae strain (*Chlorella vulgaris*) seeks to store surplus nutrients in its intracellular pools for usage during times of nutrient limitations and it is true for many algal strains (Liang *et al.*, 2009). However, at a higher CO_2 concentration (above 6%), the rate of CO_2 fixation reduces (i.e. 58mg/L/day) and this is expected since the culturing media become acidic thus put the microalgae in a harsh toxic environmental condition. The possible mechanism behind this is probably microalgae consume CO_2 in the form of bicarbonate dissolved in the media in addition to CO_2 diffusion through carbon concentrating mechanism (CCM) operation to its cell which is always the case at higher CO_2 concentration. This leads to concentration gradient between the two sides of the cell.

Moreover, as shown in Fig. 6-3, the CO_2 fixation rate at 6% CO_2 concentration, and 39^{o}C culturing temperature shows a remarkable improvement i.e. 78mg/L/day (which is in conflict with what was observed in the single factor experiment explained earlier. The simple explanation for this is that,

since the solubility of gases decreases at increased solvent temperature, hence, the media pH ceased to decrease as only moderate CO_2 concentration were able to dissolves in it. However, at lower culture temperature (25^0C) and at moderate CO_2 concentration (2%), a higher CO_2 fixation rate (175mg/L/day) was observed. James *et al.*, (1985) reported that, at lower atmospheric CO_2 concentration, carbonic anhydrase is triggered for the photosynthetic use of inorganic carbon. Moroney & Somanchi, (1999) also proved that eukaryotic microalgae's activity diminished at an elevated CO_2 concentration. In this study, the CO_2 fixation rate at ambient CO_2 concentration (i.e. 0.03%) to moderate CO_2 concentration (i.e. 4%) was higher than at extreme higher CO_2 concentration (i.e. 12%) indicating that there was a decrease in the rate of photosynthesis. Increasing temperature causes reduction in the rate of photorespiration and thereby bringing about exhaustion of carbon reserves and intracellular carbondioxide. As a result, the CO_2 fixation rate of the strain decreases with the increase of culturing temperature. Above 35°C, the carbonic anhydrase activity is drastically reduced and as a result, the low CO_2 fixation rate was observed which is in accordance with Xia & Gao., (2005) findings.

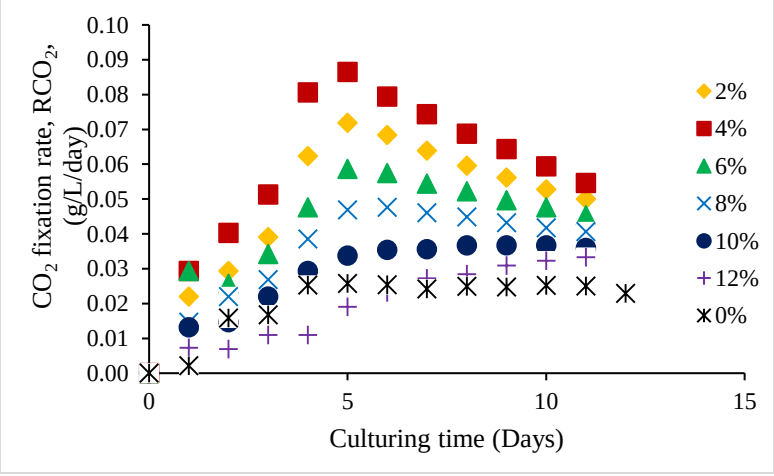


Fig. 6-3: CO_2 fix. of Cv. at different CO_2 conc.

Conditions: Temperature of 25 ± 3°C and nutrient ratio (NP) of 2:1.

Fig. 6-4**c & d** show the interaction effects of NP ratio and CO_2 concentration on the rate of CO_2 fixation rate. Here, the temperature was kept constant at 39^oC while we varied the other two factors. The nutrient ratio seems to be most influencing factor in this combination. However, no synergies were observed on these two factors with respect to the CO_2 fixation rate of the microalgae. Nevertheless, at 39^oC, the optimum values of inputs parameters are 6% CO_2 concentration and NP ratio of 1:1 to give 94mg/L/day CO_2 fixation.

Fig. 6-4**e & f** present the interaction effect of NP ratio and culturing temperature on the CO_2 fixation rate by *Chlorella vulgaris*. Here, temperature is the most influencing factor. This is expected since the microalgae metabolic activities (i.e. protein and carbohydrate synthesis) diminishes at elevated culturing temperature (Chinnassamy *et al*., 2009).

Likewise, the nutrient ratio, i.e. Nitrogen and phosphorus which is available in the form of nitrate and phosphate respectively represents two of the most crucial macroelements needed for microalgae growth. These elements have direct link with the principal metabolism of microalgae. For instance, lack of nitrogen availability results to a reduction in the generation of carbohydrates and amino acids. Its deficiency has been linked to a drastic reduction in fixation of carbon because of low production of biocatalyst which aids e^- transfer photosynthetically in the chloroplast of a vascular plant as it causes chlorophyll a pigment and hence enhances increment of carotenoids (Kumar *et al.*, 2010). Therefore, prolonging the exponential growth phase is necessary in order to obtain a higher carbon dioxide fixation rate and specific growth rate (Jin *et al.*, 2006).

Also, availability of phosphorus has been directly linked to the generation of energy required for carbon transport by producing ATP. Also, linked to the utilisation of carbon for synthesising protein through the

process of phosphorylation and thereby affecting microalgae's ability to influence CCMs (carbon dioxide concentration mechanisms) (Xu *et al.*, 2010). Xu *et al.*, (2010) studied the interacting effect of phosphorus and CO_2 concentration and he explicated that excess availability of phosphorus enhances microalgae growth due to increase in the rate of photosynthesis process. A red alga (*Gracilaria lemaneiformis*) used in the study showed its ability to be photosynthetically enhanced (by 0.072%) at higher CO_2 concentration and phosphorus level. But the study fails to view the response at a higher temperature. However, this study shows that, at 6% CO_2 concentration, the best combination is temperature of about 25^0C and nutrient ratio of 1:1 to achieve a high CO_2 fixation rate (of about 248mg/L/day).

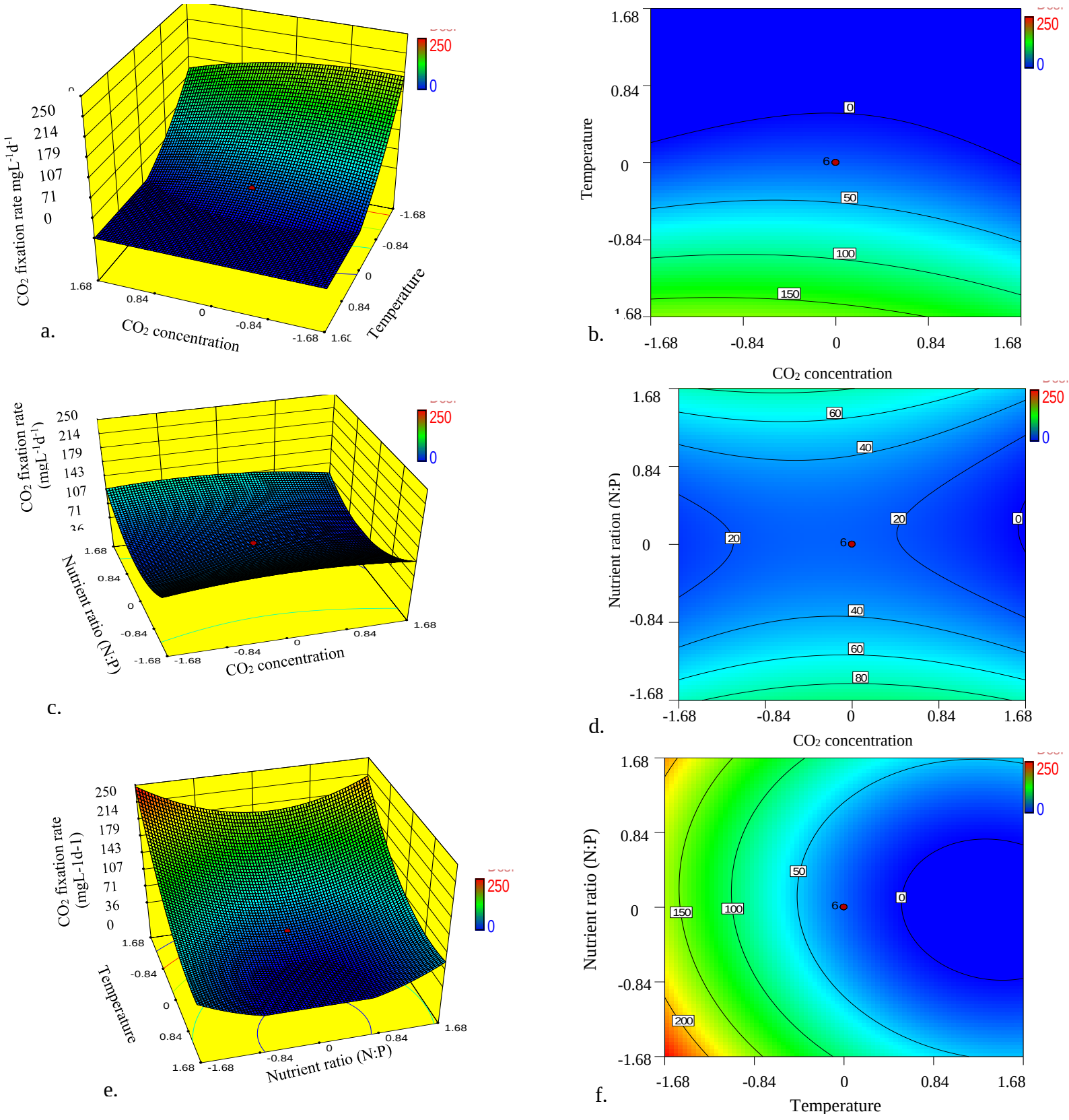


Fig. 6-4: 3D response surfaces and 2D contour lines for the CO_2 fix.

6.2.3. Synergetic Effects of Input Parameters on Biomass Productivity

Temperature is one of the main factors that affect the biomass productivity. **Fig. 6-5** a and b show the effects of culturing temperature and CO_2 concentration on biomass productivity. The increase of temperature from 25^0C results to decrease in biomass productivity which suggested that optimum temperature for biomass productivity falls below 25^0C. This is in agreement with (Kumar *et al*., 2010) who reported optimal biomass productivity at 15-26^0C for some strains including *Chlorella vulgaris*.

Fig. 6-5 c and d **s**how the interacting effect of CO_2 concentration and NP ratio on biomass productivity. Clearly, it was observed that optimal CO_2 concentration was around 5.5% while the variation of NP ratio shows very little effect on biomass productivity. **Fig. 6-5** e and f show the effect of culture temperature and NP ratio on biomass productivity of *Chlorella vulgaris*. The optimal culture temperature for biomass productivity seemed to fall below 25^0C and thereby further investigation is required.

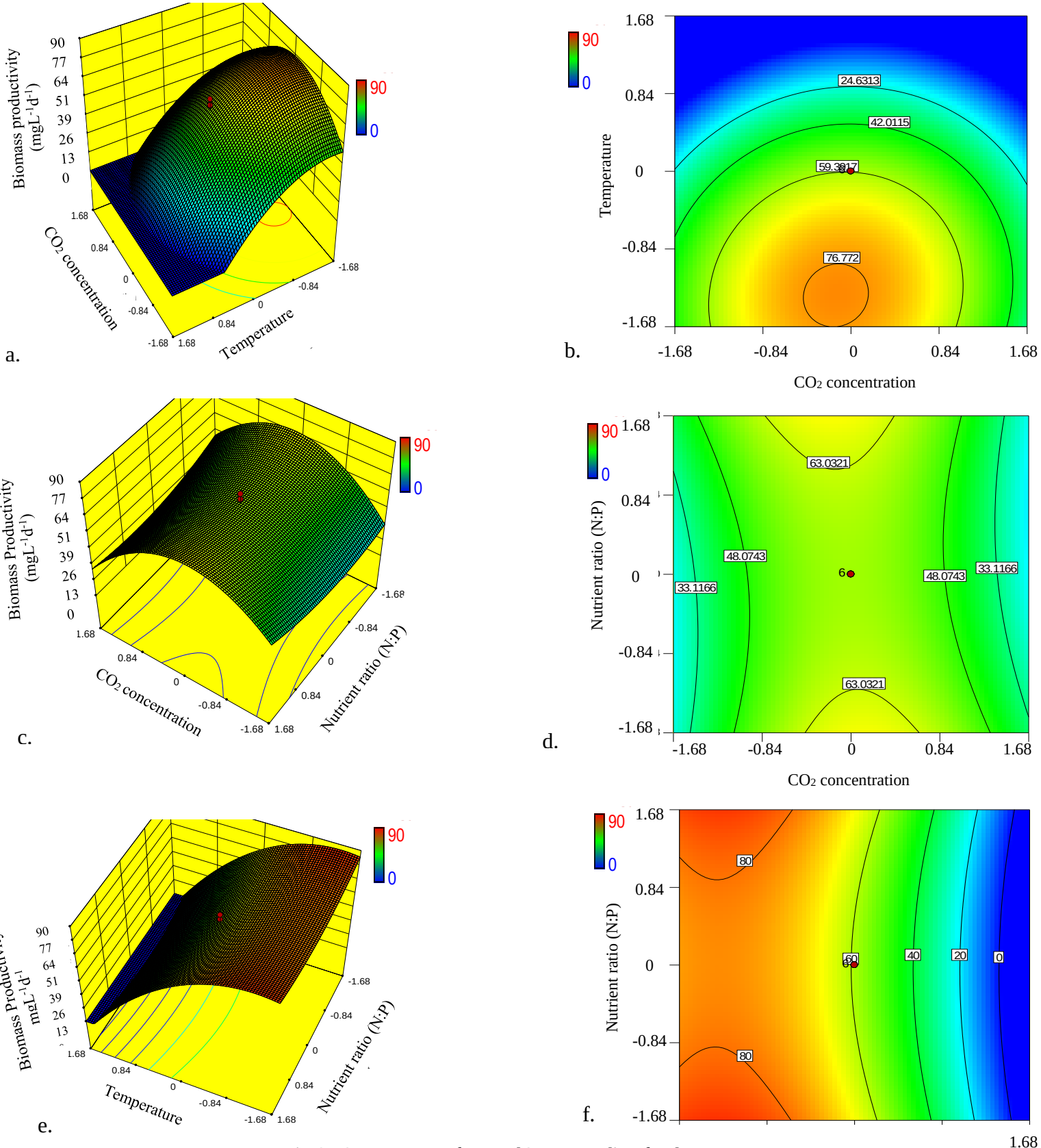


Fig. 6-5:3D response surfaces and 2D contour lines for the BP

6.3. Validation of Model

The model validation was done by comparing the model predictions with the actual experimental data by calculating the percentage errors**.** Table 6-3 represents the model predictions and experimental data for specific growth rate, CO_2 biofixation rate and biomass productivity. The specific growth rate model (Eq.(**6-1**)) shows poor agreement with experimental data since percentages (%) of error for several treatments (#1-5, 10-12, 14-16) were observed. Therefore, **Fig. 6-1**a shows low accuracy correlation between the model predictions and the experimental data.

The CO_2 fixation model (Eq.**(6-2**)) shows fine agreement with the experimental data at the intermediate levels for all three factors. Nevertheless, the high percentages (%) of error were observed at treatments that have a very low CO_2 concentration (0% CO_2, #3) and very high CO_2 concentration (12% CO_2, #12) as shown in Table 6-3. Therefore, it was deduced that the model developed could not be employed when dealing with either extremely low or high treatments (i.e. extreme corner points -α and α). However, **Fig. 6-1**b clearly presents a strong correlation between the model predictions and the experimental data.

The biomass productivity model (Eq.**(6-3**)) shows satisfactory agreement with experimental data at intermediate levels for all three factors similar to the CO_2 fixation model Table 6-3. The inaccuracy of the model was also observed at treatment with either low CO_2 (0% CO_2, #3) or very high CO_2 concentration (12% CO_2, #12). **Fig. 6-1**c illustrates also a satisfactory correlation between the model predictions and the experimental data.

All the models showed poor predictions in some treatments since these models are quadratic and hence could not probably describe the dynamic behavior of algal growth and CO_2 biofixation in

all kinetic regimes. Hence, comprehensive mathematical models need to be developed for further study.

Table 6-3: The Model Prediction and The Corresponding Experimental Data.

Experimental number	Specific growth rate (l/day)			CO_2 fixation rate (mg/L/day)			Productivity (mg/L/day)		
	Model prediction	Experimental data	Error (%)	Model Prediction	Experimental data	Error (%)	Model prediction	Experimental data	Error (%)
1	0.10 ± 0.39	0.7	37.84	112.09 ± 6.7	117.33	4.46	66.00 ± 10.0	63.54	3.82
2	1.40 ± 0.38	1.86	27.16	93.66 ± 6.4	94.91	1.32	66.60 ± 10.0	76.36	12.78
3	1.00 ± 0.38	1.5	33.02	13.10 ± 6.4	8.46	54.88	25.32 ± 10.0	34.64	26.9
4	-0.63 ± 0.38	0	0	-12.65 ± 6.4	0	0	-17.76± 10.0	0	-
5	0.92 ± 0.39	0.63	46.72	127.4 ± 6.7	131.27	2.95	67.40 ± 10.0	61.47	9.65
6	1.88 ± 0.20	1.88	0.29	23.25 ± 3.3	22.12	5.13	59.10 ± 0.00	55.72	6.08
7	1.88 ± 0.20	1.9	1.21	23.25 ± 3.3	23	1.09	59.10 ± 0.00	56.00	5.54
8	0.40 ± 0.39	0	-	10.66 ± 6.7	0	-	13.07 ± 10.0	0.00	-
9	0.37 ± 0.39	0	-	4.46 ± 6.7	0	-	9.26 ± 10.0	0.00	-
10	1.26 ± 0.39	1.04	21.88	91.04 ± 6.7	91.2	0.18	61.27 ± 10.0	53.36	14.84
11	1.20 ± 0.39	0.94	27.46	117.64 ± 6.7	127.69	7.87	72.00 ± 10.0	69.87	3.05
12	1.03 ± 0.38	1.46	29.55	-9.48 ± 6.4	4.4923	31.15	21.18 ± 10.0	33.20	36.21
13	0.40 ± 0.39	0	-	5.85 ± 6.7	0	-	12.77 ± 10.0	0.00	-
14	1.57 ± 0.38	1.99	21.3	80.23 ± 6.4	80.12	0.14	67.28 ± 10.0	79.03	14.89
15	1.88 ± 0.20	1.63	15.15	23.25 ± 3.3	25	7	59.10 ± 0.00	55.50	6.49
16	0.53 ± 0.38	0.82	35.91	166.46 ± 6.4	154.91	7.45	76.43 ± 10.0	80.04	4.51
17	0.43 ± 0.39	0	-	0.78 ± 6.7	0.8	2.84	7.28 ± 10.0	0.00	-
18	1.88 ± 0.20	1.93	2.49	23.25 ± 3.3	25	7	59.10 ± 0.00	66.00	10.45
19	1.88 ± 0.20	1.88	0.29	23.25 ± 3.3	22.12	5.13	59.10 ± 10.0	62.00	4.67
20	1.88 ± 0.20	1.88	0.29	23.25 ± 3.3	22.12	5.13	59.10 ± 10.0	55.72	6.08

(Mean ± 95% CI)

6.4. Optimization

6.4.1. Specific Growth Rate

The optimum specific growth rate was calculated by using the quadratic model presented in Eq. (6-1). The maximum specific growth rate was predicted to be 1.93 ± 0.19 d^{-1} at optimal values of 34^{o}C, 4:1 NP ratio, and 6% CO_2 concentration and the optimization plot is shown in Fig. 6-6a.

6.4.2. CO_2 Fixation Rate

The CO_2 fixation rate model (Eq.(6-2)) was used to estimate the maximum CO_2 fixation rate. The CO_2 fixation rate was calculated to be 251.9 ± 13.5 $mgL^{-1}d^{-1}$ at an optimal set of 4% CO_2, 1:1 NP

ratio and 25^{O}C culture temperature, respectively and the optimization plot is shown in Fig. 6-6b. Generally, increase in phosphate concentration increases the rate of phosphorus consumption by microalgae thereby producing more chlorophyll pigment to enhance its specific growth rate which in turn increase the rate of CO_2 biofixation (Cho *et al.*, 2015). Hence, CO_2 biofixation rate was optimized at around elevated phosphate levels (i.e. nutrient ratio 1:1) and culturing temperature (25^{O}C). Besides, it is well known that microalgae cell luxuriously consumes and store the excess CO_2 which consequently results in higher CO_2 biofixation rate at around 4-6% CO_2 concentration.

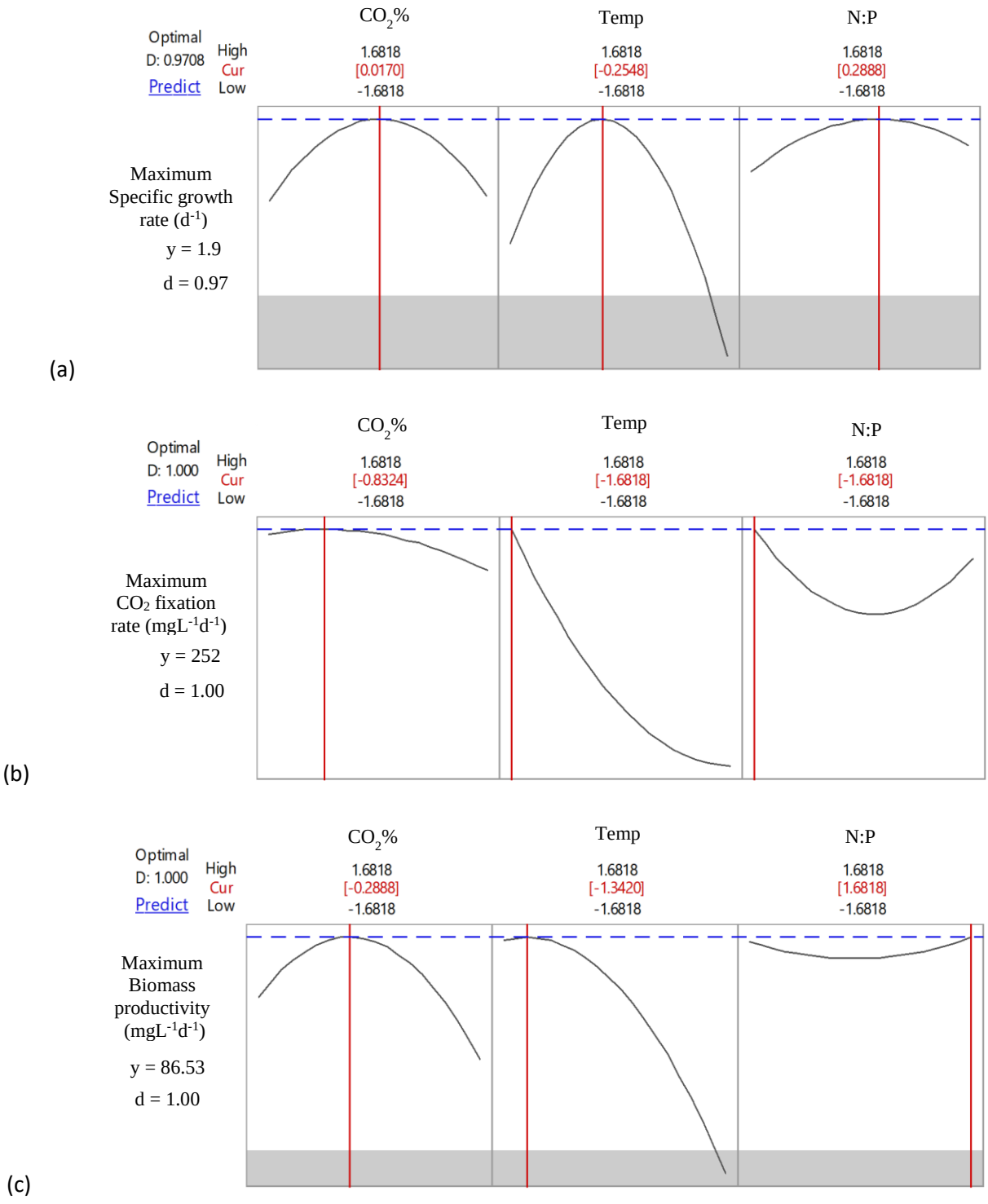


Fig. 6-6: Optimization plot for (a) SG, (b) CO_2 fix. and (c) BP

6.4.3. Biomass Productivity

The maximization of the biomass productivity was done by employing the biomass productivity quadratic regression model (Eq.(6-3)) to estimate the optimum set of factors investigated in the considered range. The optimum CO_2 concentration, NP ratio and culture temperature were estimated to be 4.8%, 8:1 and 28^{O}C respectively and maximum biomass productivity was predicted to be 86.5 ± 20.0 $mgL^{-1}d^{-1}$ and the optimization plot is shown in Fig. 6-6c.

6.5. Multi-Objective Optimization

The response optimizer plot was employed to optimize the CO_2 biofixation rate and biomass productivity concurrently. The CO_2 fixation rate of 182.84 ± 8.42 $mgL^{-1}d^{-1}$ and a biomass productivity of 78.5 ± 10.0 $mgL^{-1}d^{-1}$ were obtained at the solution set of 4% initial CO_2 concentration, 6:1 NP ratio and 25^{o}C culturing temperature with composite desirability of 0.99 as shown in Fig. **6-7**. The results are reasonably in agreement with (Chinnasamy *et al.*, 2009) findings. However, it may not feasible to compare the results in the literature, because of the different media, conditions in which the strain was cultivated.

6.6. Validation of Optimal Points

To validate the optimal model predictions of the CO_2 fixation rate, biomass productivity, and multi-objective optimized points, set of duplicate experiments were conducted for each case. Table 6-4 shows the model predictions and actual experimental values for all three optimal points. The Maximum prediction error of 15.5% was obtained at optimized solutions of CO_2 biofixation rate for biomass productivity. These prediction errors are reasonably low since the 95% confidence intervals (CIs) were employed for both the CO_2 biofixation rate and biomass productivity.

Therefore, a strong agreement was found between the experimental data and model prediction data and thus, the developed models can effectively be employed for prediction of biomass productivity and CO_2 biofixation of *Chlorella vulgaris*.

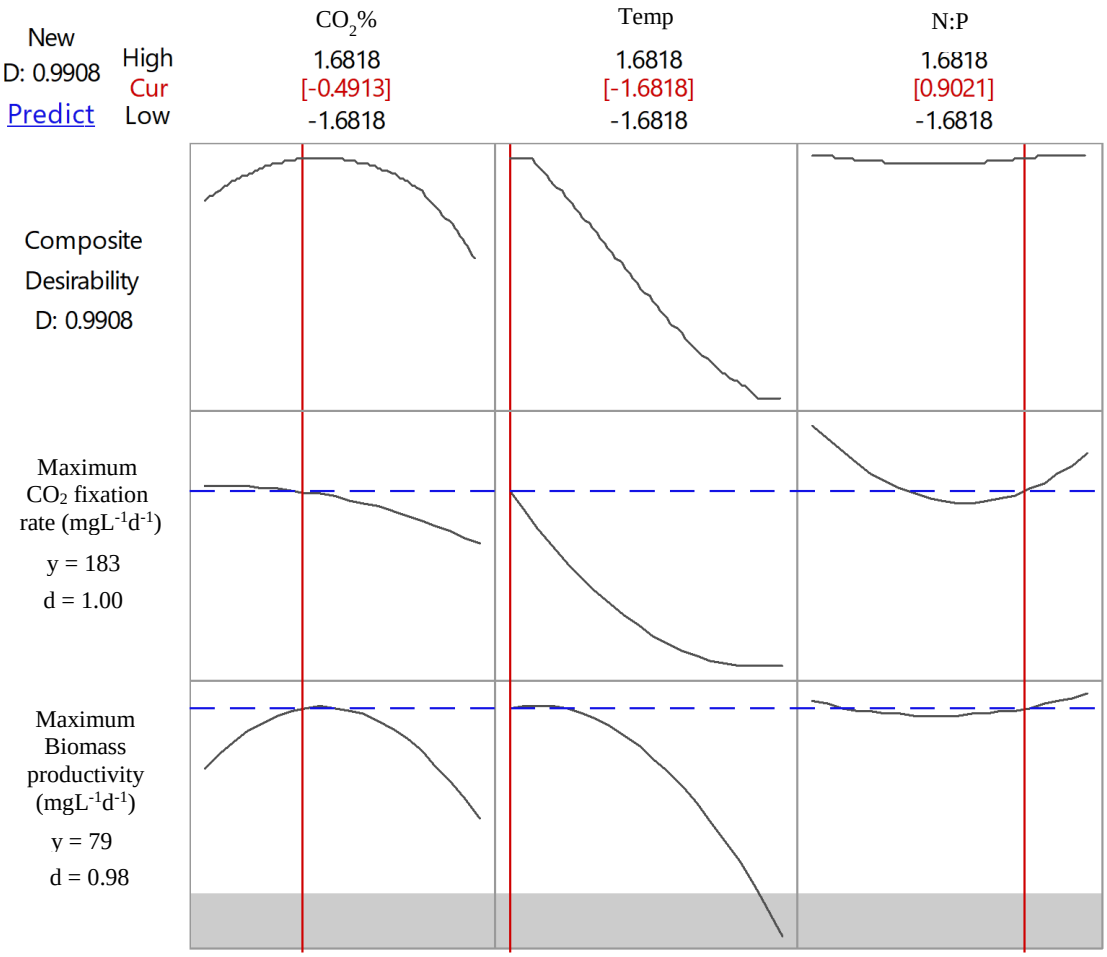


Fig. 6-7: The Multi-objective optimization plot for CO_2 fix. and BP.

Table 6-4: Optimum Level for Both Single and Multi-Objective Optimization.

Objective function	**Optimal condition**			**CO_2 fixation rate ($mgL^{-1}d^{-1}$)**			**Productivity ($mgL^{-1}d^{-1}$)**		
	CO_2 (%)	NP ratio	Culture temperature (^oC)	Model prediction	Experimental data	Error (%)	Model prediction	Experimental data	Error (%)
CO_2 fixation rate	4	1:1	25	251.9 ± 13.5	246.70	2.1	76.07 ± 20.0	90.0	15.5
Productivity	4.8	8:1	28	182.3 ± 10.1	181.1	0.7	86.53 ± 15.0	88.1	1.8
Multiobjective optimization	4	6:1	25	182.8 ± 8.4	179.1	2.1	78.58 ± 12.5	83.4	5.8

(Mean ± 95% CI)

CHAPTER 7

OPTIMIZATION OF CAPTURE AND WASTEWATER TREATMENT BY *CHLORELLA VULGARIS*.

Herein, we report the concurrent utilisation of microalgae (*Chlorella vulgaris*) for fixation of CO_2 and wastewater treatment by employing response surface method.

7.1. Model Development

To demonstrate the relationships among the three response parameters (e.g., CO_2 fixation rate, nitrogen and phosphorus removal efficiencies), multiple regression analysis was employed by varying the set of input parameters (e.g., CO_2 concentration, the NP ratio, and culturing temperature). Experimental data obtained from central composite design (CCD) presented in **Table** 7-2 are used in the development of the following quadratic regression equations to adequately depict CO_2 fixation rate, nitrogen and phosphorus removal efficiencies of *Chlorella vulgaris*.

$$CO_2 \text{ Fixation rate (g/l/day)} = 0.02325 - 0.00418\ x_1 - 0.05331\ x_3 - 0.00400\ x_2 - 0.00608\ x_1^2 + 0.01901\ x_3^2 + 0.02257\ x_2^2 + 0.00630\ x_1x_3 - 0.00282\ x_1x_2 + 0.00371\ x_2x_3 \quad \textbf{(7-1)}$$

$$\text{Efficiency of nitrogen removal (\%)} = 47.71 - 1.75\ x_1 - 40.93\ x_3 - 1.96\ x_2 - 10.64\ x_2^2 + 0.30\ x_3^2 + 12.09\ x_2^2 - 0.27\ x_1x_3 + 0.27\ x_1x_2 + 0.31\ x_2x_3 \quad \textbf{(7-2)}$$

$$\text{Efficiency of phosphorus removal (\%)} = 34.78 - 4.21\ x_1 - 26.20\ x_3 + 13.99\ x_2 - 7.56\ x_2^2 + 1.63\ x_3^2 + 0.59\ x_2^2 + 1.86\ x_1x_3 - 1.15\ x_1x_2 - 14.80\ x_2x_3 \quad \textbf{(7-3)}$$

Fig. 7-1 a-c shows the plots of experimental data versus model predictions for specific growth rate, CO_2 fixation rate, nitrogen removal efficiency and phosphorus removal efficiency respectively. The coefficient of determination (i.e. R^2) of the quadratic regression models for the specific growth rate, CO_2 fixation rate, nitrogen removal efficiency and phosphorus removal efficiency were 0.99, 0.95 and 0.99 respectively showing that the regression models developed can effectively depict the relationship between the investigated factors and responses.

ANOVA test as shown in **Table** 7-1 was employed to determine the p-values by fitting the experimental data to the quadratic regression models i.e. Eqn. (7-1)-(7-3) (Ogunnaike, 2010). Here, the analysis is considered to be significant when the p-value in the model term is less than (<) 0.05. As shown in **Table** 7-1, the p-value for the culturing temperature of all responses is equal to 0.00 (i.e. p-value = 0.00) which indicate that culturing temperature is most influencing factor in comparison to all other response parameters. This is in accordance with Al Ketife et al., (2016) findings. The CO_2 fixation rate, nitrogen removal efficiency and phosphorus removal efficiency were also significantly influenced by the CO_2 concentration as the p-value were 0.02, 0.01, and 0.02 respectively. There was a synergetic effect of NP ratio and culturing temperature on Phosphorus removal efficiency as the p-value = 0.00. Also, there exist a synergetic effect of culturing temperature and CO_2 concentration on CO_2 fixation rate as the interaction effect p-value = 0.045. The analysis of these interacting effects is presented in proceeding sections.

Table 7-1. ANOVA For (A) % P Removal, (B) % N_2 Removal, and (C) CO_2 Fix

(a)

Term	Coefficient (β)	Standard error	t-Value	p-Value
Constant	34.78	2.35	14.79	0.00 ς
x_1	-4.21	1.56	-2.70	0.02 ς
x_3	-26.20	1.56	-16.79	0.00 ς
x_2	13.99	1.56	8.96	0.00 ς
x_1^2	-7.56	1.52	-4.97	0.00ς
x_3^2	1.63	1.52	1.07	0.31ς
x_2^2	0.59	1.52	0.39	0.71
x_1x_3	1.86	2.04	0.91	0.38
x_1x_2	-1.15	2.04	-0.57	0.58
x_2x_3	-14.80	2.04	-7.26	0.00

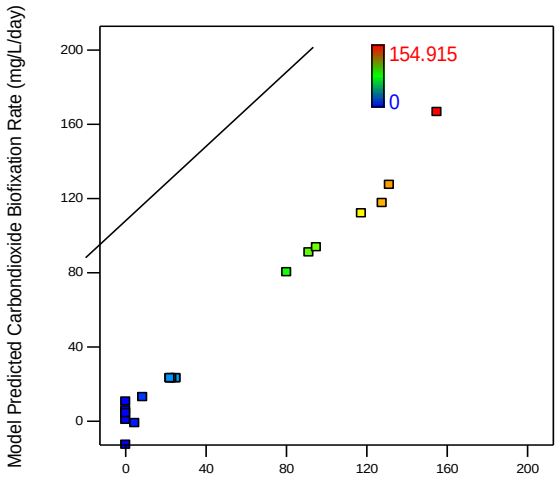
(b)

Term	Coefficient (β)	Standard error	t-Value	p-Value
Constant	47.71	5.06	9.43	0.00ς
x_1	-1.75	3.36	-0.52	0.61
x_3	-40.93	3.36	-12.20	0.00ς
x_2	-1.96	3.36	-0.58	0.57
x_1^2	-10.64	3.27	-3.26	0.01ς
x_3^2	0.30	3.27	0.09	0.93
x_2^2	12.09	3.27	3.70	0.00ς
x_1x_3	-0.27	4.38	-0.06	0.95
x_1x_2	0.27	4.38	0.06	0.95
x_2x_3	0.31	4.38	0.07	0.94

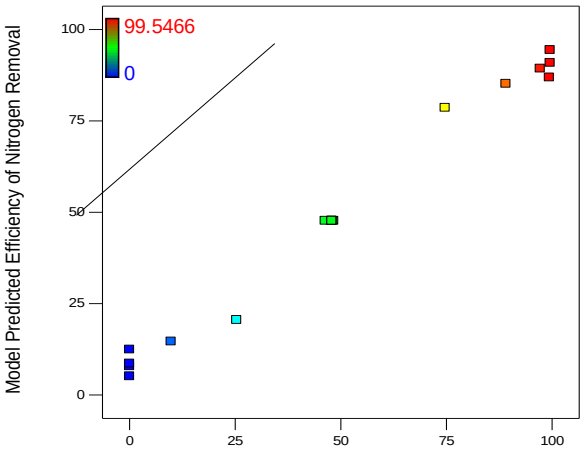
(c)

Term	Coefficient (β)	Standard error	t-Value	p-Value
Constant	0.02	0.003	6.95	0 ς
x_1	-0.00	0.002	-1.89	0.09
x_3	-0.05	0.002	-24.03	0.00 ς
x_2	0.00	0.002	-1.80	0.10
x_1^2	0.01	0.002	-2.82	0.02 ς
x_3^2	0.02	0.002	8.81	0 ς
x_2^2	0.02	0.002	10.45	0.00 ς
x_1x_3	0.01	0.003	2.18	0.05 ς
x_1x_2	0.00	0.003	-0.97	0.35
x_2x_3	0.001	0.003	1.28	0.23

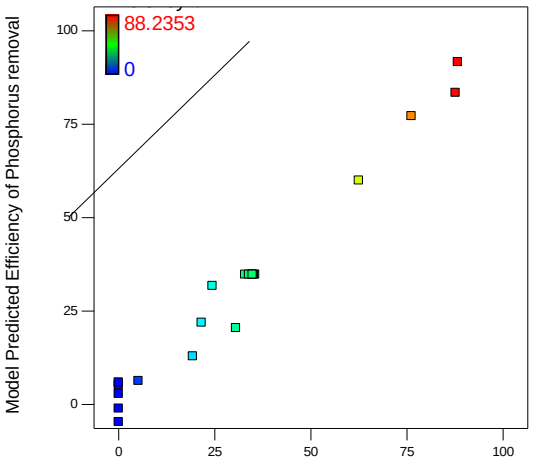
ς Significant at p-value < 0.05



(a) Experimental Carbondioxide Biofixation Rate (mg/L/day) **(R^2 = 0.987)**



(b) Experimental Efficiency of Nitrogen Removal **(R^2= 0.95)**



(c) Experimental Efficiency of Phosphorus Removal **(R^2 = 0.99)**

Fig. 7-1: Model Validation (a) (b) % of N_2 removal (c) % of p removal

Table 7-2. SG, CO_2 Fix., % of N_2 Removal, And % of P Removal

Experimental number	CO_2 Fixation rate (mg/L/day)	Efficiency of nitrogen removal (%)	Efficiency of phosphorus removal (%)
1	117.33	99.55	21.58
2	94.91	89.10	19.30
3	8.46	25.37	30.53
4	0.00	0.00	0.00
5	131.27	99.54	24.41
6	22.12	47.90	34.83
7	23.00	47.92	33.90
8	0.00	0.00	0.00
9	0.00	0.00	0.00
10	91.20	99.37	76.18
11	127.69	97.21	88.24
12	4.49	9.85	5.16
13	0.00	0.00	0.00
14	80.12	74.71	62.47
15	25.00	48.32	35.20
16	154.91	97.14	87.63
17	0.00	0.00	0.00
18	25.00	46.30	32.89
19	22.12	47.90	35.50
20	22.12	47.90	34.83

7.2. Analysis of Synergetic Effects

In order to achieve better apprehension of the results, quadratic regression models Eq. (7-1)-(7-3) developed were employed to generate three – dimensional (3D) response surfaces and two – dimensional (2D) contour plots, which are presented in Fig. 7-2 and Fig. 7-3. The results showed synergetic effects with the several combinations of input factors on CO_2 fixation rate, nitrogen and

phosphorus removal efficiencies. It is important to note that both surface and contour plots were plotted for 2 factors and the remaining factor was fixed at a centre level.

However, only a few synergies effect was observed as explained from the ANOVA table. Phosphorus removal efficiencies were altered by the combined effect of NP ratio and culturing temperature. And the CO_2 fixation rates were affected by the combined effect of CO_2 concentration and culturing temperature which have been explained in chapter 6. No combined effect of any two factors were observed on Nitrogen removal efficiencies.

7.2.1. Synergetic Effect of Input Parameters on Nitrogen Removal Efficiency.

Fig. 7-2**a-f** show the interacting effect of input parameters on nitrogen removal efficiency. Nitrogen removal efficiency follows an identical trend of being enhanced with decreasing culture temperature from 60^0C to 25^0C as shown in Fig. 7-2**a, b, e & f** with almost 100% removal at relatively low temperature (25^0C). However, no synergies were observed on any two factors with respect to the Nitrogen removal efficiency of the microalgae. On the other hand, it apparently seems to reduce at very low and very high CO_2 concentration as shown in Fig. 7-2**c & d**. This is suggested to be true since at moderate CO_2 concentration (i.e. 6%), the enzyme nitrogen reductase is being activated thereby aid in the assimilation of nitrogen and also generating high concentration of bicarbonate via addition of CO_2 and protons produced from microalgae cells by consuming nitrogen and phosphorus (Judd et al., 2015; Redfield, 1963). This, however, maintains a benign condition for biomass growth by keeping the pH at a neutral level. Hence, large biomass production results in nutrients depletion. The best combination was observed to be temperature of 33^OC, 6% CO_2 concentration and NP ratio of 1:1 for 98% Nitrogen removal.

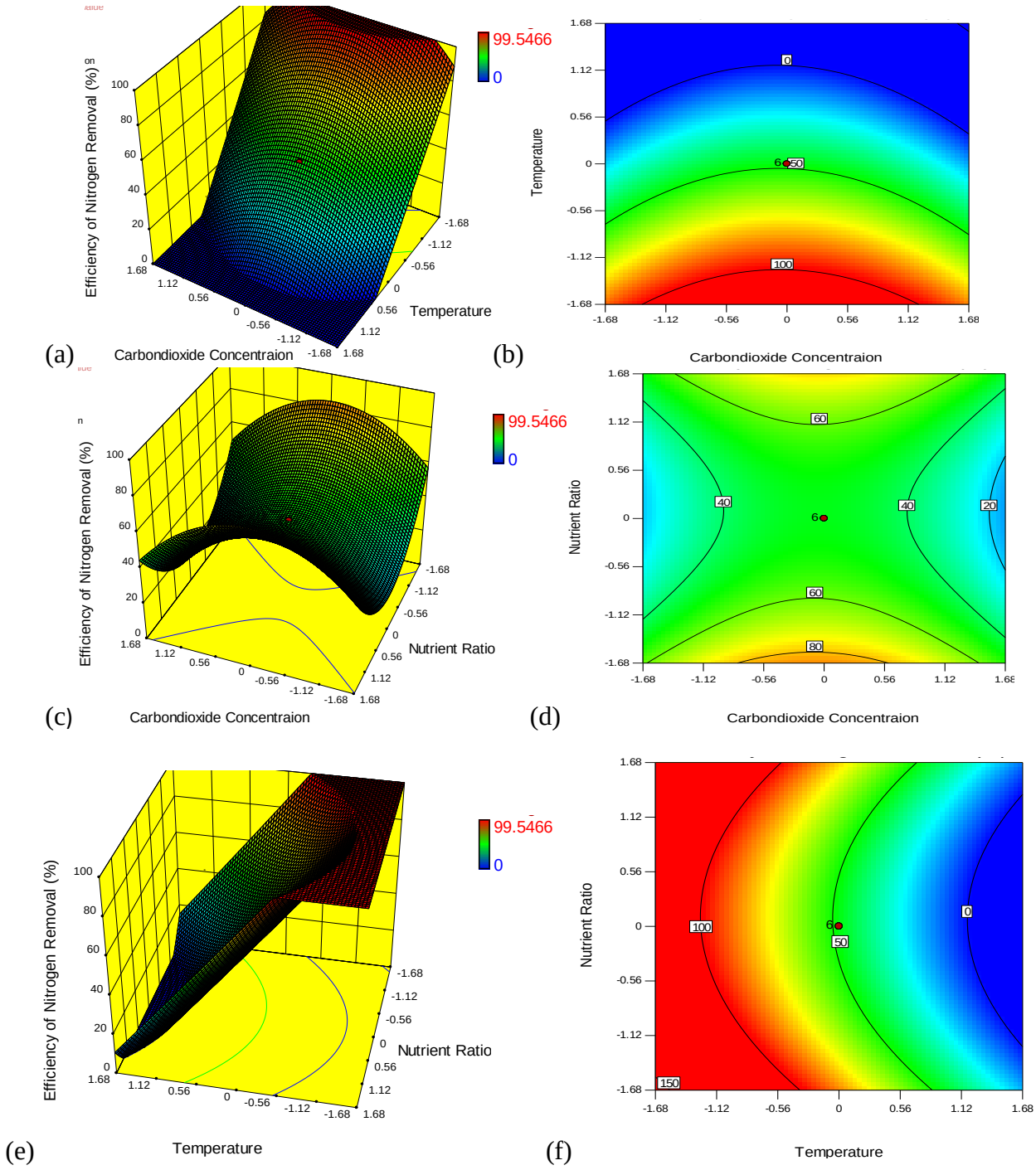


Fig. 7-2: 3D response surfaces and 2D contour lines for the % removal of N_2

7.2.2. Synergetic Effect of Input Parameters on Phosphorus Removal Efficiency.

Fig. 7-3**a-b** represent the interacting effect of temperature and CO_2 concentration on phosphorus removal efficiency. Culturing temperature still appears as the main influencing parameter. However, no synergies were observed between these factors.

Fig. 7-3**c-d** shows the interacting effect of CO_2 concentration and NP ratio. Also, no synergetic effect of these two factors were observed on phosphorus removal efficiencies.

Fig. 7-3**e & f** presents the interacting effect of temperature and NP ratio on the phosphorus removal efficiency. Here, synergies were observed as the p-value is equal to 0.00 as shown on **Table** 7-1. Phosphorus removal efficiency showed a flatter response at low temperature and relatively low phosphorus concentration (i.e. NP, 8:1). Thermal damage associated with high culture temperature results in cell death or low biomass concentration which consequently reduce the removal efficiency. Since depletion of phosphorus by microalgae cells is linked with consumption of dissolved inorganic carbon via photosynthesis, removal efficiency of phosphorus is more influenced by change in pH than nitrogen removal through abiotic precipitation, albeit microalgae removal mechanism of phosphorus remains the same (Su et al., 2012). Hence at elevated culturing temperature, solubility of CO_2 decreases which consequently reduced the amount of dissolved inorganic carbon available for photosynthesis and as well increase the pH of the media. However, the best combination of these parameters is 4% CO_2 concentration, 31^{o}C culturing temperature and 1:1 NP ratio to achieve a 99.9% phosphorus removal efficiency.

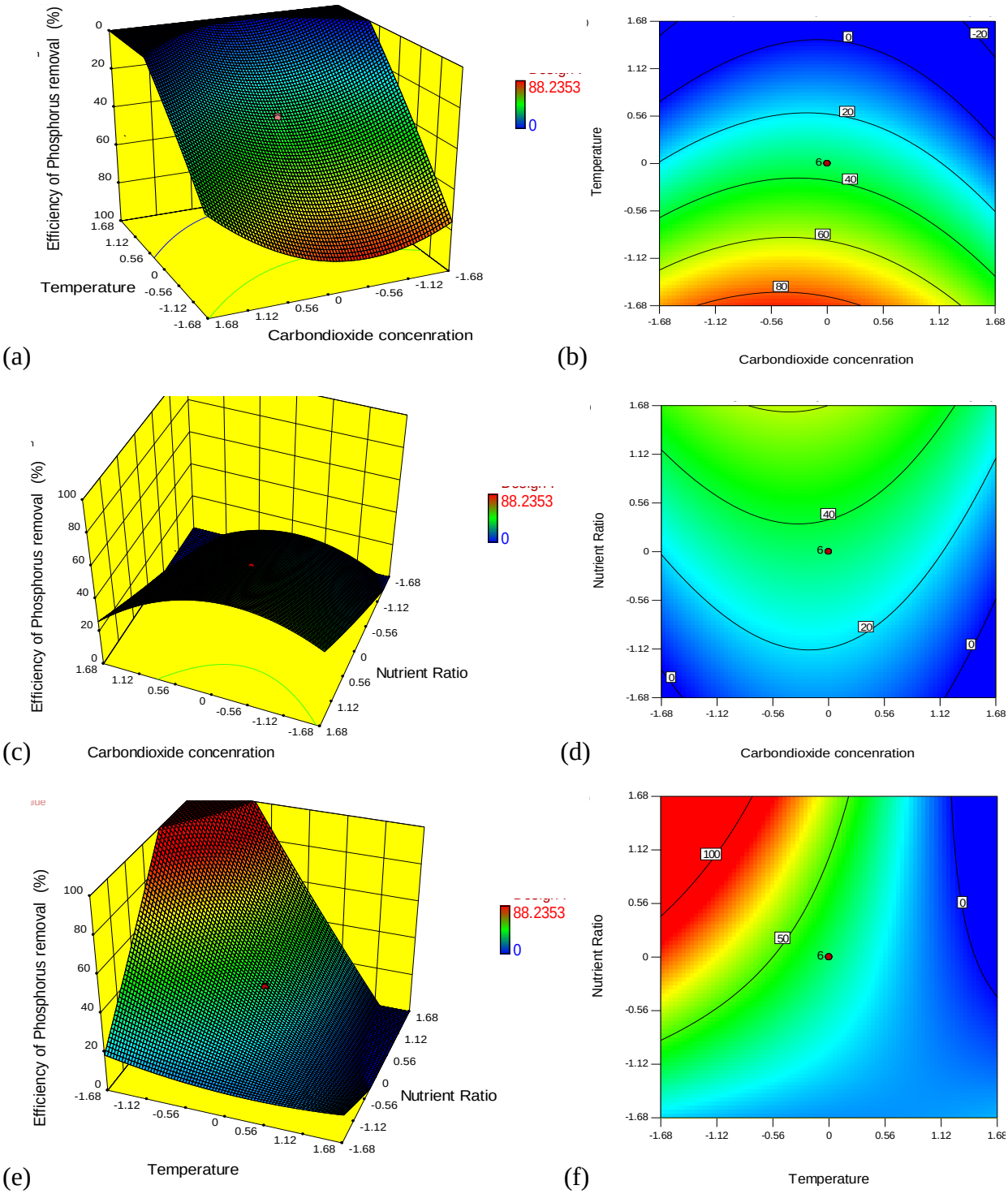


Fig. 7-3: 3D response surfaces and 2D contour lines for the % removal of P

7.3. Multi-Objective Optimisation

The response optimizer plot was employed to optimize the CO_2 fixation rate, nitrogen removal efficiency and phosphorus removal efficiency, simultaneously. The maximum CO_2 fixation rate of 127.7 ± 5.1 $mgL^{-1}day^{-1}$, nitrogen removal efficiency of 99.9 ± 7.7% and phosphorus removal efficiency of 88.5 ± 3.6% were obtained at the solution set of 4% initial CO_2 concentration, 7:1 NP ratio and 28°C culturing temperature with composite desirability of 1.0 as shown in Fig. 7-4.

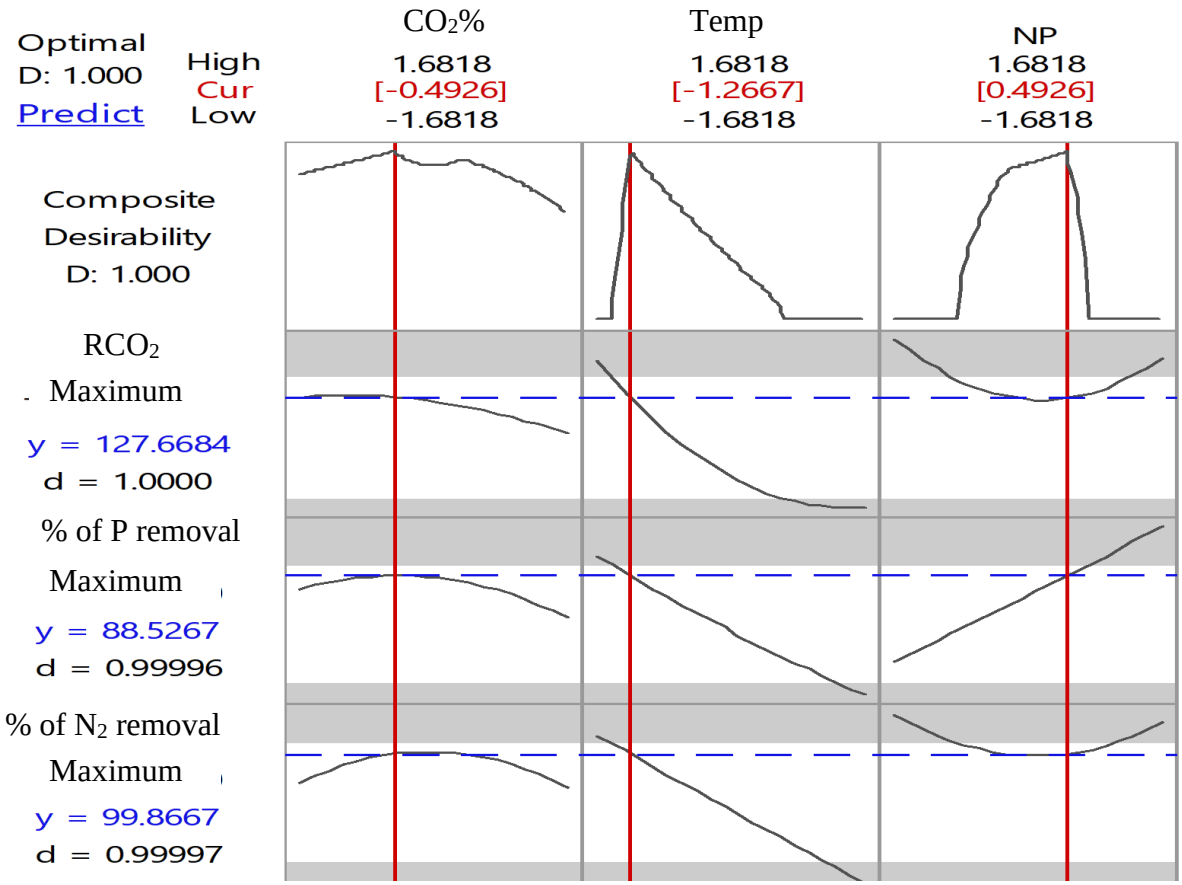


Fig. 7-4: The Multi-objective optimization plot for CO_2 fix, % of N_2 removal, and % of P removal

7.4. Validation of Optimal Points

To validate the optimal model predictions of the multi-objective optimised points, set of duplicate experiments were conducted. **Table 7-3 s**hows the model predictions and actual experimental values for the three optimal points. The maximum errors between model predictions and experimental values for CO_2 fixation rate, nitrogen removal efficiency and phosphorus removal efficiency were 0%, 4.6% and 11.5%, respectively. These errors are reasonably low since the 95% confidence intervals (CIs) were employed. Overall, a strong agreement was found between the experimental and model prediction data and thus, the developed models can effectively be employed for prediction of CO_2 fixation and wastewater treatment efficiency of *Chlorella vulgaris.*

Table 7-3. Optimum Level and Corresponding Experimental Data

Factors	Optimal conditions	Response	Actual	Model prediction	Error (%)
CO_2	4%	CO_2 fixation rate (mg/L/day)	127.7	127.7 ± 5.1	0
NP ratio	7:1	% of N_2 removal	95.2	99.9 ± 7.7	4.6
Temperature	28^{o}C	% of P removal	100	88.5 ± 3.6	11.5

(Shown as Mean Value 95% Confidence Interval)

CHAPTER 8

JUSTIFICATION FOR ACCEPTING THE REGRESSION MODELS.

There are two sample data for each response, one obtained from the experiment of *Neo Oleoabundans* and the other from the models generated. The experiment uses a factorial design varying 3 factors (each of 5 levels) and as such, it requires 5^3 or 125 individual runs. But to avoid such large number of runs, response surface method was employed which statistically suggest how the experiment could be partly implemented without using all treatments (Toktas et al., 2014).

However, in this section, a full factorial study was performed on the experiment to determine if the generated models are good predictors for the experiment responses and as such, if they are deemed good enough, they would be used to generate all missing values responses.

Basically, the main aim for comparing the model and the experimental data is to see if the model and experimental data do not differ significantly based on the 15 sample points. And as such we can extend our problem to a factorial design which can be analyzed using Anova. In this subsection, the goal is to justify why the models were accepted.

Below is the summary of the test.

- 15 distinct set of data that were obtained by running the experiment were collected.
- The treatment combinations were noted.
- For each response, there exist the model value and the experimental value.
- Normality test was first carried out on each sample pair in other to see which type of test would be suitable for comparing the pairs.

- Then, two-sample test was carried out for each response by comparing the experimental and model values to determine whether statistically equivalent or not.

Details of the tests carried out and the subsequent conclusions are presented in this chapter. $\alpha = 0.05$ (as mention in the objective section), for all the tests.

8.1 Hypothesis

Throughout this study, we shall assume 5% level of significance for the test of our hypotheses i.e. $\alpha = 0{:}05$.

Factor A: ***C*O₂ (%) (denoted *c*)**

If we let μ_i; $i = \{1, \ldots, 5\}$ be the mean effect of the ith level (treatment) of CO_2% on the response variables, then:

Null Hypothesis (H_0): $\mu_1 = \ldots = \mu_5$. In other words, $C0_2$ has no significant effect on the response variables.

Alternative Hypothesis (H_1): At least one $\mu_i \neq 0$. In other words, CO_2 has significant effect on the response variables.

Factor B: Nutrient ratio (denoted *n*)

If we let μ_i; $i = \{1, \ldots, 5\}$ be the mean effect of the ith level (treatment) of Nutrient ratio on the response variables, then:

Null Hypothesis (H_0): $\mu_1 = \ldots = \mu_5$. In other words, Nutrient ratio has no significant effect on the response variables.

Alternative Hypothesis (H_1): At least one $\mu_i \neq 0$. In other words, Nutrient ratio has significant effect on the response variables.

Factor C: Temperature (denoted *t*)

If we let μ_i; $i = \{1, \ldots, 5\}$ be the mean effect of the *i*th level (treatment) of temperature on the response variables, then:

Null Hypothesis (H_0): $\mu_1 = \ldots = \mu_5$. In other words, temperature has no significant effect on the response variables.

Alternative Hypothesis (H_1): At least one $\mu_i \neq 0$. In other words, temperature has significant effect on the response variables.

Interaction effects: AB, AC, BC.

Null Hypothesis (H_0): For any given interaction effect, the null hypothesis states that, the interaction factors under consideration has no significant effect on the response variables.

Alternative Hypothesis (H_1): The given interaction factor has effect on the responses.

Response: Specific Growth Rate (Denoted as *SG*)

For the specific growth, the values obtained from the experiment and model are shown in the table below with their specific treatment combinations.

$$SG = 0.918 + x_1 c - x_2 t + x_3 n + x_4 c^2 - x_5 t^2 + x_6 n^2 - x_7 ct + x_8 cn + x_9 tn \qquad 8\text{-}1$$

Where,

$$x_1 = 0.2471, \quad x_2 = 0.4509, \; x_3 = 0.0902, \; x_4 = 0.116, \; x_5 = 0.2216,$$
$$x_6 = 0.0934, \; x_7 = 0.059, \; x_8 = 0.097, \; x_9 = 0.147.$$

Table 8-1: Response values for Specific Growth Rate

Treatments (*c*,*n*,*t*)	**Experimental**	**Model**
(10, 2:1, 32)	1.9597	1.7443
(12, 4:1, 39)	1.5776	1.4740
(2, 2:1, 50)	0	0.2483
(2, 6:1, 32)	1.1345	1.0167
(2, 2:1, 32)	1.1345	0.9793
(2, 6:1, 50)	0	0.3857
(6, 4:1, 39)	0.9057	0.9220
(6, 1:1, 39)	1.0966	1.0818
(0, 4:1, 39)	1.0162	0.8837
(10, 6:1, 32)	1.7623	1.6817
(6, 4:1, 60)	0	0
(10, 6:1, 50)	0	0.3227
(6, 4:1, 25)	0.6806	1.1010
(6, 8:1, 39)	1.3657	1.1446
(10, 2:1, 50)	0	0.2853

Both the experimental and model data follow the normal distribution at $\alpha = 0.05$. Outcomes of the normality tests for each sample are shown in Fig. 8-1 and Fig. 8-2 respectively.

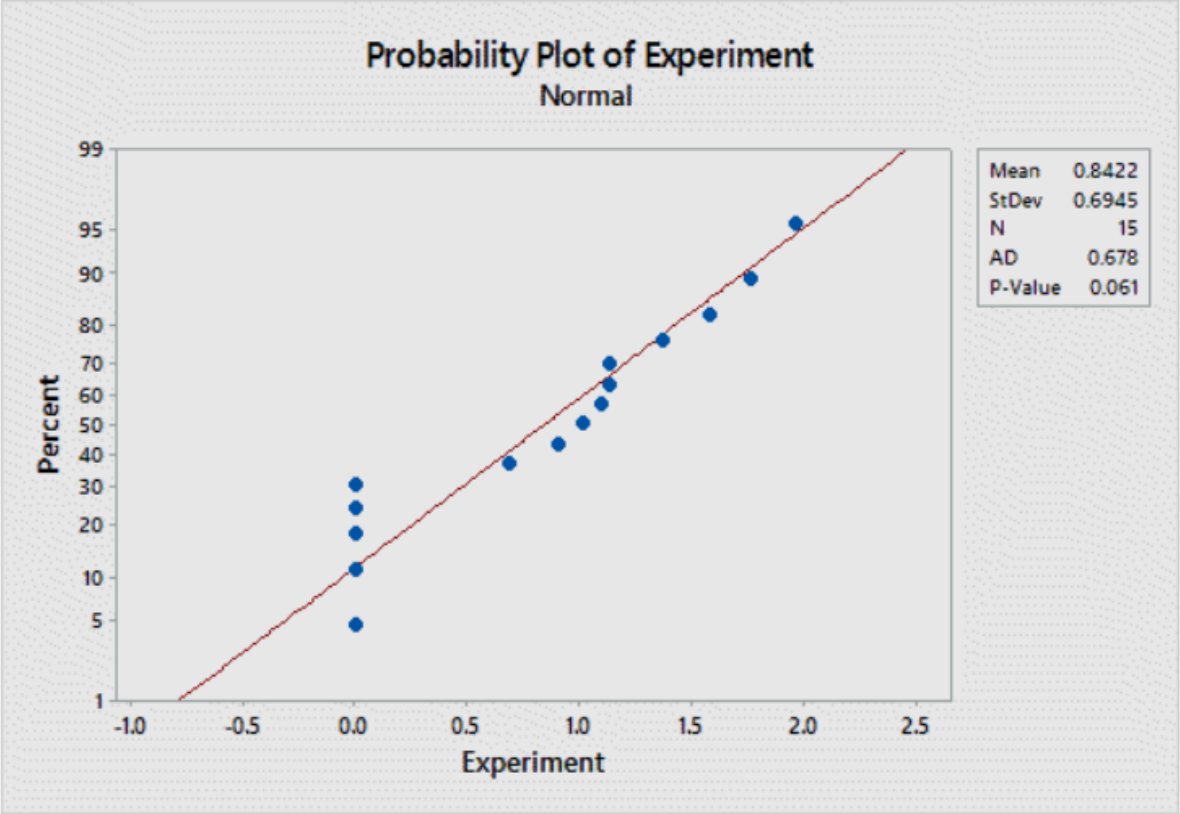


Fig. 8-1: Normality test for the model specific growth data

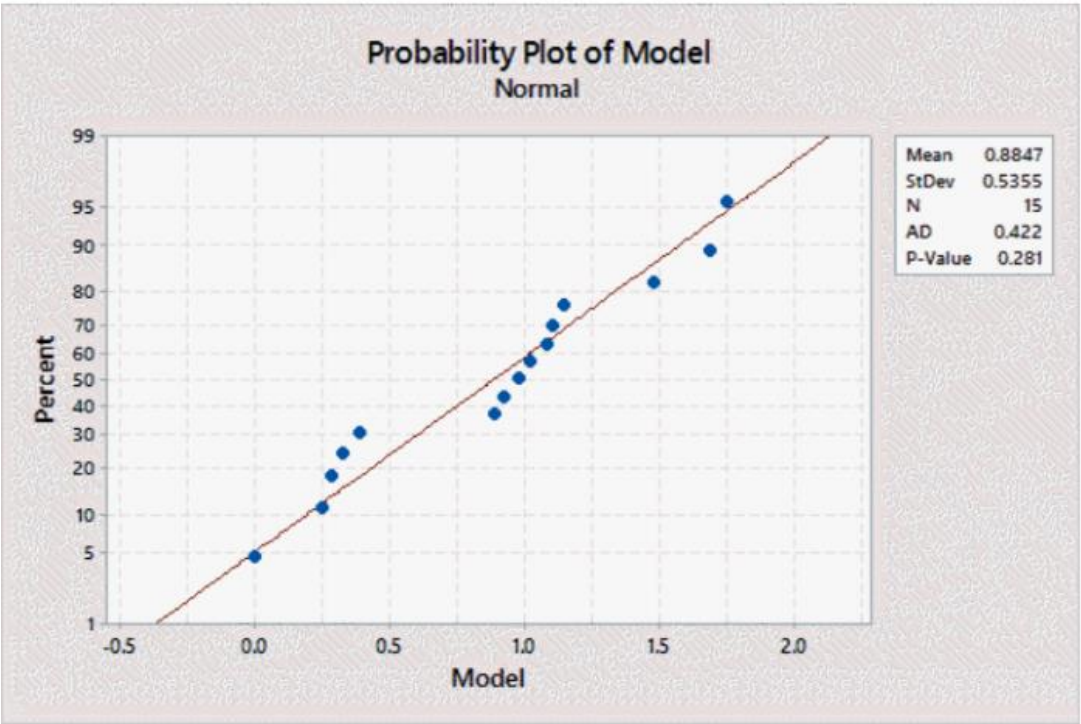


Fig. 8-2: Normality test for the experimental specific growth data.

After running the *t*-test (assuming equal variance, this was verified using the 2-variance test on Minitab as shown in Fig. 8-3), a *p*-value of $p = 0{:}853$ was obtained. Minitab output of the test is shown in Fig. 8-4. since $p = 0{:}853 > \alpha$, it can be concluded that the experimental and model data do not differ significantly. As such we accept the model Eq. **8-1** as a good predictor for the specific growth rate response in our experiment.

Response: Maximum Biomass Productivity (Denoted as *MP*)

For Maximum Productivity, the values obtained from the experiment and model are shown in the Table 8-2, together with the specific treatment combinations.

$$MP = 16.19 + y_1 c - y_2 t - y_3 n + y_4 c^2 + y_5 t^2 + y_6 n^2 + y_7 ct - y_8 cn + y_9 tn \quad \text{8-2}$$

Where,

$$y_1 = 0.680,\ y_2 = 32.360,\ y_3 = 4.140,\ y_4 = 7.550,\ y_5 = 10.130,$$
$$y_6 = 0.560,\ y_7 = 3.620,\ y_8 = 2.940,\ y_9 = 2.480$$

Table 8-2: Response Values for Maximum Productivity.

Treatments (*c*,*n*.,*t*)	**Experimental**	**Model**
(10, 2:1, 32)	82.13	73.41
(12, 4:1, 39)	42.08	39.69
(2, 2:1, 50)	0.00	0.00
(2, 6:1, 32)	86.69	66.05
(2, 2:1, 32)	84.85	73.41
(2, 6:1, 50)	0.00	0.00
(6, 4:1, 39)	16.13	17.19
(6, 1:1, 39)	21.83	23.35
(0, 4:1, 39)	19.30	37.40
(10, 6:1, 32)	60.43	54.29
(6, 4:1, 60)	0.00	0.00
(10, 6:1, 50)	0.00	1.77
(6, 4:1, 25)	76.00	100.26
(6, 8:1, 39)	0.00	9.42
(10, 2:1, 50)	0.00	10.97

The result of the normality test shows that, the experimental data does not follow the normal

distribution at $\alpha = 0{:}05$. Outcomes of the normality tests for each sample are shown in Fig. 8-3 and Fig. 8-4 respectively.

Mann-Whitney Test was then carried out on the data, a p-value of $p = 0{:}8347$ was obtained. Minitab output of the test is shown in Minitab Sequence 3. From the result of this comparison, since $p = 0{:}8008 > \alpha$, it can be concluded that the experimental and model data are statistically similar, in other words they do not differ significantly. As such, the model Eq. **8-2** can be accepted as a good predictor for the maximum productivity response.

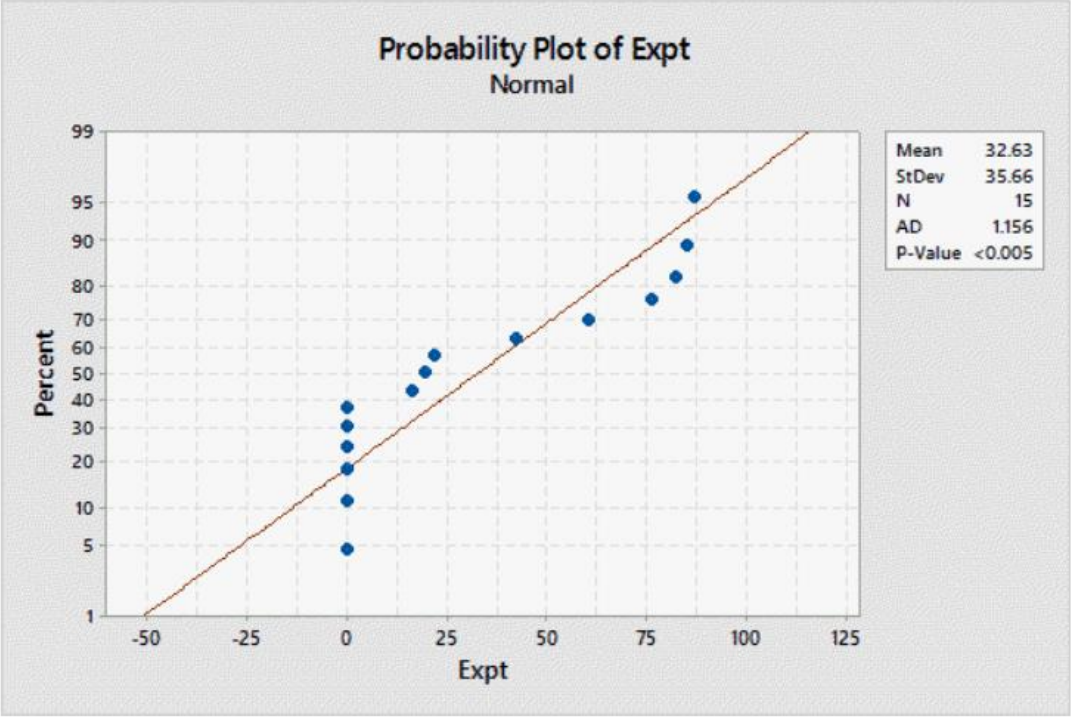


Fig. 8-3: Normality test for the model Maximum Productivity data.

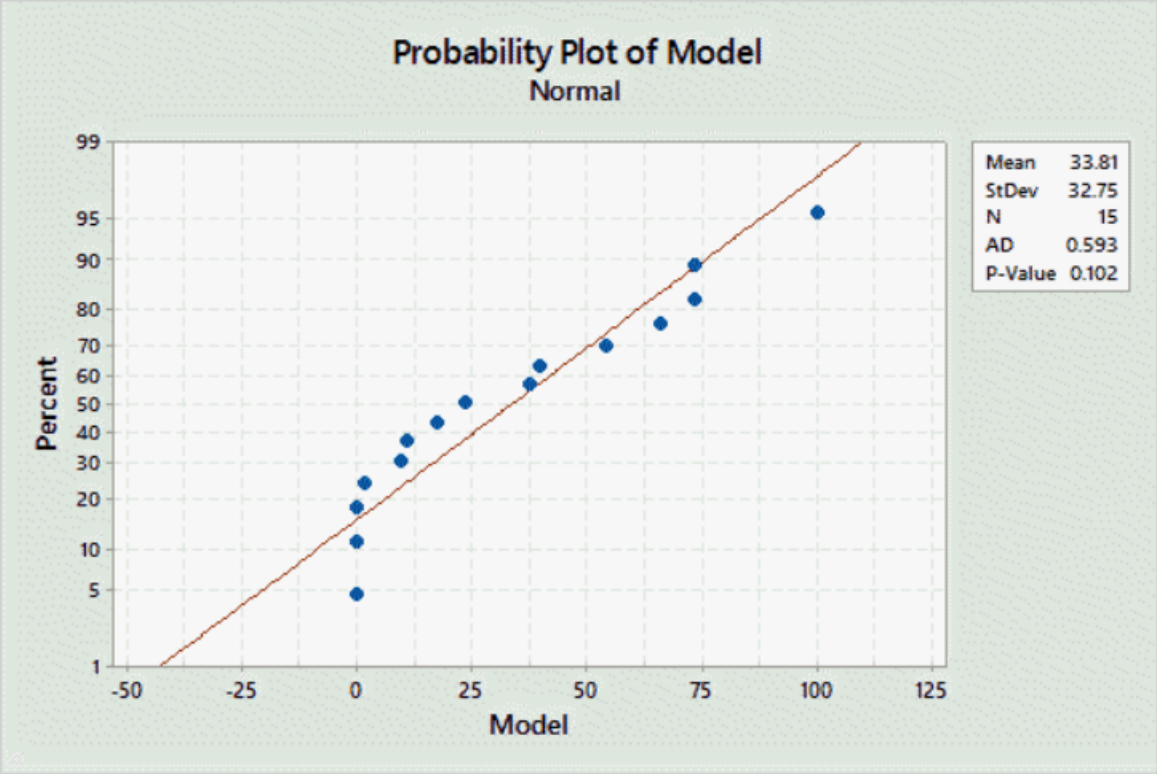


Fig. 8-4: Normality test for the experimental Maximum Productivity data.

Response: Biofixation

For the CO_2 biofixation, the values obtained from the experiment and model are shown in the table below with their specific treatment combinations.

$$BF = 25.47 + z_1c - z_2t - z_3n + z_4c^2 + z_5t^2 + z_6n^2 + z_7ct - z_8cn + z_9tn \quad \textbf{8-3}$$

Where,

$$z_1 = 1.08,\ z_2 = 51.12,\ z_3 = 0.33,\ z_4 = 10.17,\ z_5 = 14.26,\ z_6 = 8.05,$$
$$z_7 = 5.72,\ z_8 = 4.65,\ z_9 = 3.92$$

Table 8-3: Response Values for Biofixation.

Treatments (*c*,*n*,*t*)	Experimental	Model
(10, 2:1, 32)	129.73	113.33
(12, 4:1, 39)	66.47	56.05
(2, 2:1, 50)	0.00	0.00
(2, 6:1, 32)	136.94	114.11
(2, 2:1, 32)	134.03	113.31
(2, 6:1, 50)	0.00	8.27
(6, 4:1, 39)	25.49	25.47
(6, 1:1, 39)	34.49	48.79
(0, 4:1, 39)	30.49	52.42
(10, 6:1, 32)	95.46	95.53
(6, 4:1, 60)	0.00	0.00
(10, 6:1, 50)	0.00	12.57
(6, 4:1, 25)	120.06	151.78
(6, 8:1, 39)	50.48	47.68
(10, 2:1, 50)	0.00	14.69

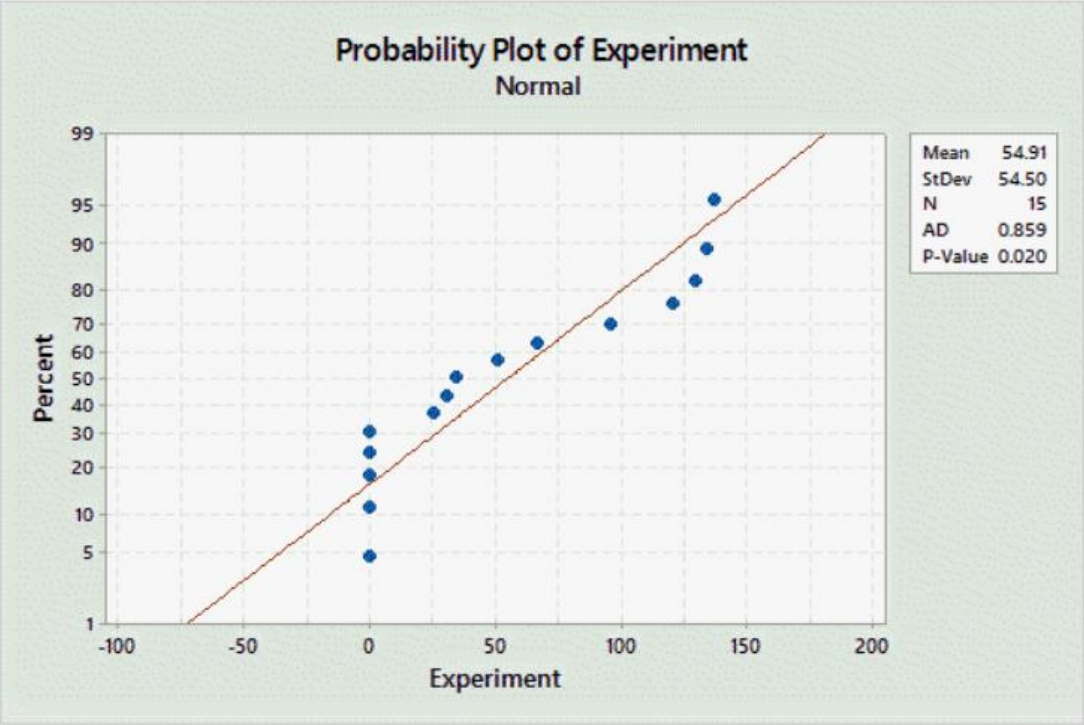


Fig. 8-5: Normality test for the model biofixation data.

The result of the normality test shows that, the experimental data does not follow the normal distribution at $\alpha = 0.05$. Outcomes of the normality tests for each sample are shown in Fig. 8-5 and Fig. 8-6 respectively.

Nonparametric 2-sample test was then carried out on the data, a *p*-value of $p = 0{:}8347$ was obtained. Minitab output of the test is shown in Fig. 8-6. From the result of this comparison, since $p = 0{:}8347 > \alpha$, it can be concluded that the experimental and model data are statistically similar, in other words they do not differ significantly. As such, the model 8-3 can be accepted as a good predictor for the CO_2 fixation rate.

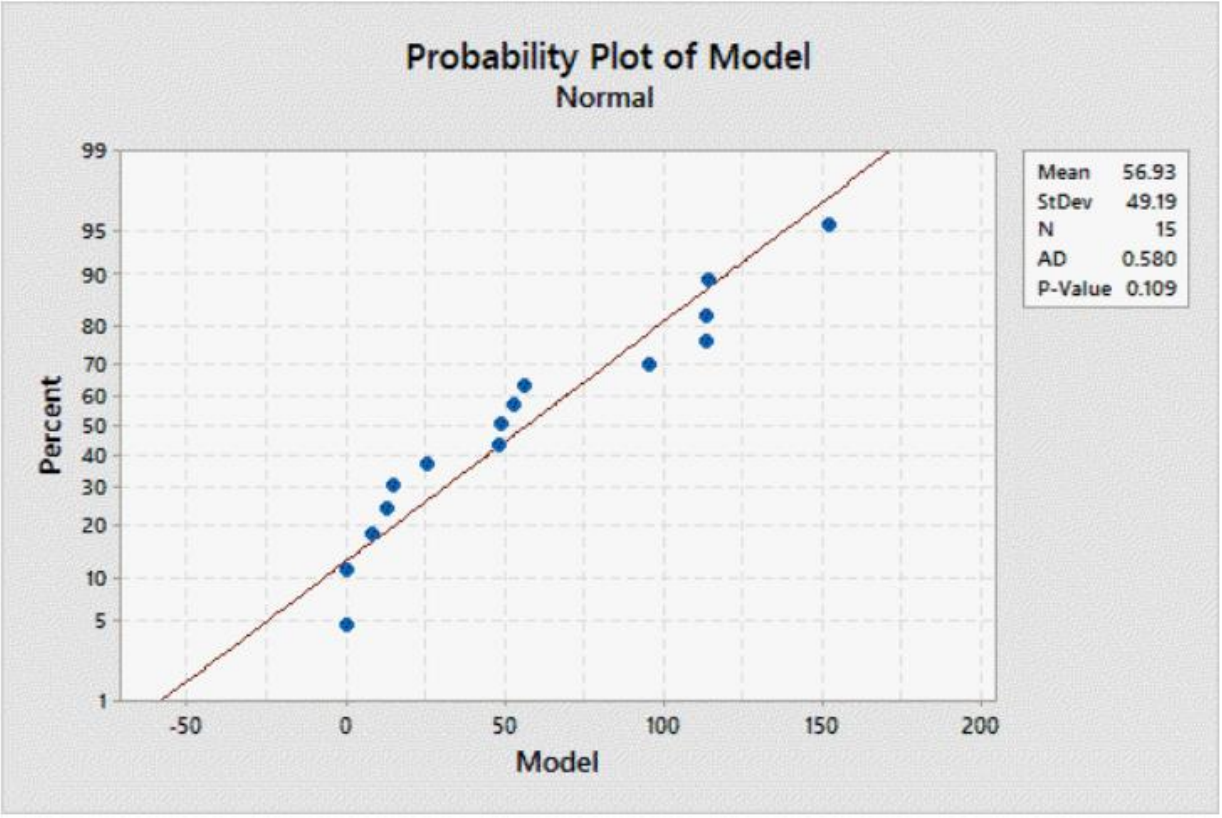


Fig. 8-6: Normality test for the experimental biofixation data

Following all the tests conducted in this section, it has been proved that the responses obtained from the models in Eq. **8-1**, **8-2**, and 8-3 do not differ significantly from the responses observed

from the actual experiment. Hence, the models in Eq. **8-1**, **8-2**, and 8-3 can be accepted as good predictors for the response values.

8.2. Empirical Relation and Data Layout

It has been established that the design technique follows the completely randomized design and for each treatment combinations applied to an experimental unit, there are 3 responses. In the cases of the univariate Anova for a 3-factor factorial design, i.e. A with $1 \leq i \leq a$ level, B with $1 \leq j \leq b$ levels, C with $1 \leq k \leq c$ levels and $1 \leq l \leq r$ replicates, we have the following empirical relation between the response (dependent variable) and the independent variables (treatments):

$$yijkl = \mu + \tau i + \beta j + \gamma k + (\tau\beta)ij + (\tau\gamma)ik + (\beta\gamma)jk + (\tau\beta\gamma)ijk + \epsilon ijkl \quad \textbf{8-4}$$

In Manova for 3 independent variables context, the same structure for the empirical formula as in Eq. 8-4 would be maintained. But the variables (both dependent and independent) in 7 are all scalers. Therefore, switching to Manova would make all the variables become $n \times 1$ vectors.

Table 8-4: Responses Captured as Vectors

$\boldsymbol{t_1}$	$\boldsymbol{t_2}$	....	$\boldsymbol{t_{243}}$
$y_1 = \begin{bmatrix} y_{11} \\ y_{12} \\ y_{13} \end{bmatrix}$	$y_2 = \begin{bmatrix} y_{21} \\ y_{22} \\ y_{23} \end{bmatrix}$	....	$y_{125} = \begin{bmatrix} y_{243\,1} \\ y_{243\,2} \\ y_{243\,3} \end{bmatrix}$

8.2.1. Non-Normality Problem

Prior the analysis, there is need to point out a very major challenge which made taking a detour an option in the analysis. As concluded earlier from previous analysis, the three responses (specific growth rate, maximum productivities and CO_2 biofixation rate) were all not normally distributed. This posed a very big challenge, because for the multivariate factorial experiment, the

nonparametric tests analyses were nontrivial. As such, Johnson's transformation was employed in the normalization of the data.

The Johnson's transformation was useful for normalizing only CO_2 biofixation rate at α = 0.04, with a transformation shown in Eq. 8-5:

$$0.473044 + 0553767 \times \ln\left[\frac{X + 5.34992}{221.973 - X}\right] \qquad \textbf{8-5}$$

But the other two responses weren't normalized by Johnson's at α = 0:04. Normal Score was then employed for the other 2 responses. We assume a definition of *Normal Score* which relates to assigning alternative values to data points within a dataset, with the broad intention of creating data values than can be interpreted as being approximations for values that might have been observed had the data arisen from a standard normal distribution.

The normal score gave a normalization of maximum biomass productivities response data although at α = 0.01, while specific growth rate was still not normalized even subject to other transformations. Following the difficulty posed by this normality, the first detour taken was setting α = 0.01 for the Manova since they were only able to be normalized at α = 0*:* 01. Hence, Manova was employed for the two normalized responses i.e. CO_2 biofixation and maximum biomass productivities. While nonparametric factorial analysis was employed for to specific growth rate. The Fig. 8-7 below shows the Johnson's transformation of CO_2 biofixation rate response while the normality test for maximum biomass Productivities response which shows normality at α = 0:01 is shown in Fig. 8-8.

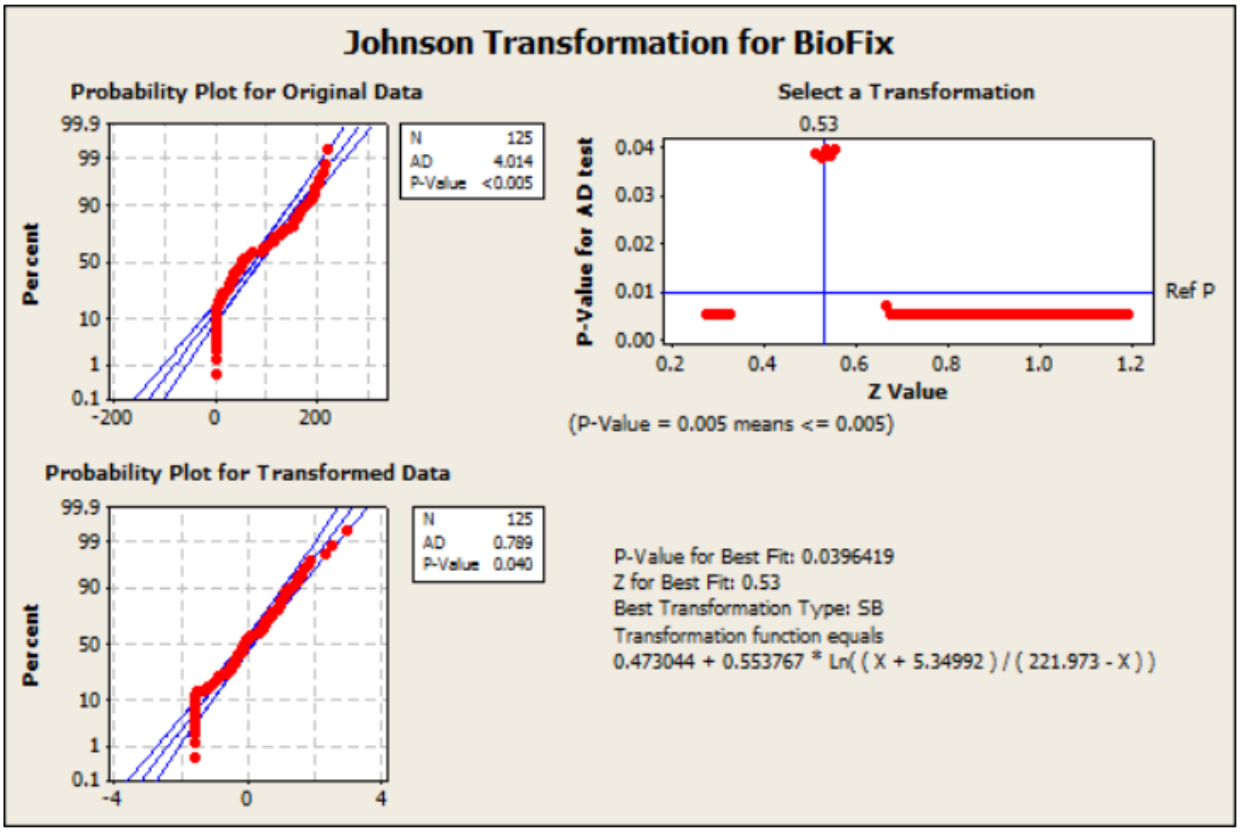


Fig. 8-7: Johnson's Transformation for CO_2 biofixation rate.

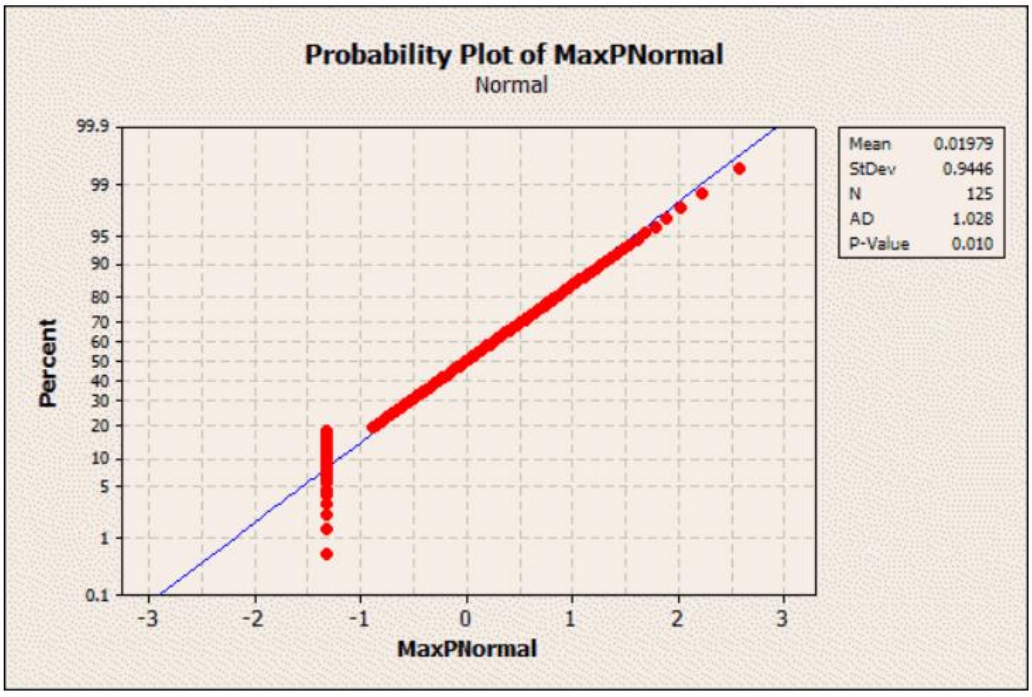


Fig. 8-8: Normality Test for Normal Score of maximum Productivities

Notations: In Minitab, we shall have the following conventions for conveniences:

- **Biofix** stands for the rate of CO_2 biofixation and **BiofixNormal** is for the Normalized CO_2 Biofixation rate data.
- **SG** represents the specific growth rate.
- **MaxP** stands for maximum biomass productivities and **MaxPNormal** is for Normalized maximum biomass Productivity data.

8.2.2. Conclusions from Manova

Wilks', Lawley-Hotelling, Pillai's, Roy's all make their observations based on their different criterion. And as it is in the literature, non-seems to be a bad yardstick. So, we won't be losing generality if we choose one of these 4 and base our decision making on it. Among these, Lawley-Hotelling criterion was employed.

Factor A: For all the single factors, it was observed that, the corresponding *p*-values satisfy $p<\alpha$, hence, null hypothesis can be rejected in each case of the single factors. Hence, it can be concluded that nutrient ratio, culturing temperature and CO_2 concentration all have significant effects on the responses investigated.

8.2.3. Plots and Analysis of Residuals

From the two residual plots, i.e. CO_2 biofixation rate and Maximum Biomass Productivities, the normal probability plots of each of the two response variables all show normality and independence because almost all the data in the case of the two data plots lie along the line. And especially the data around the center of the plots. Of course, the awareness of their normality has been established since transformations were done, but the plots serve as an added advantage to drive home the claim.

About the constant variance, since the plot of residual vs fitted values in the two cases seem not have a pattern and as such, it can be concluded that the constant variance assumption is valid. Although this assumption is tricky to establish.

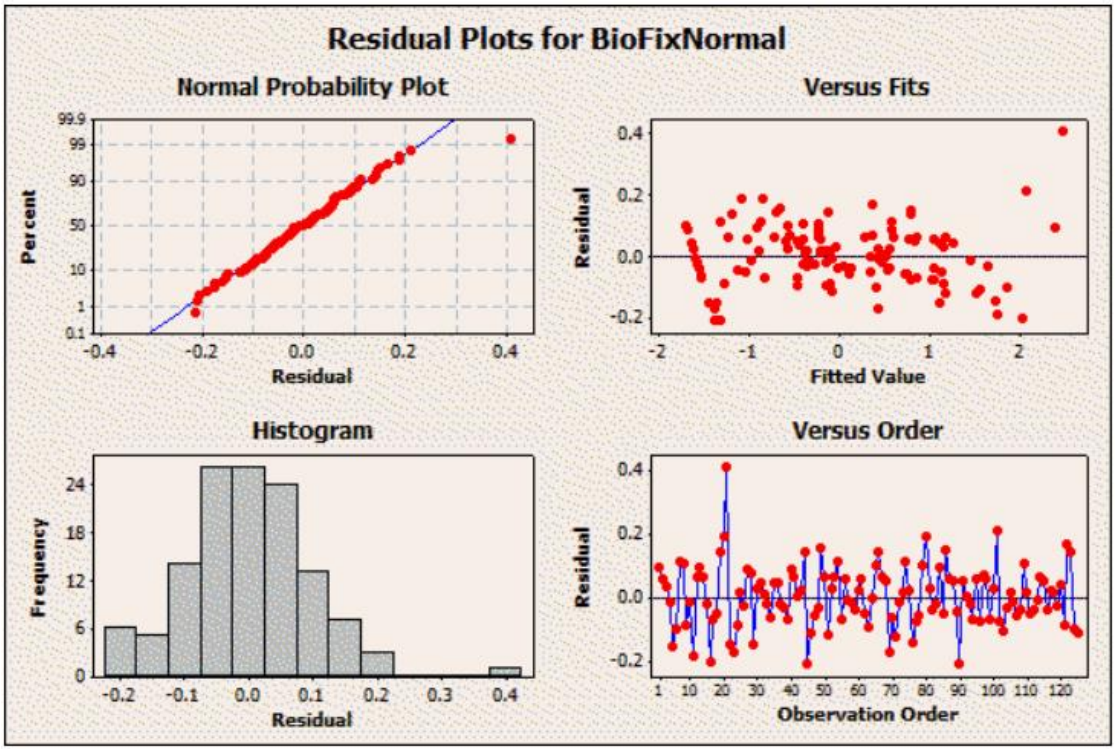


Fig. 8-9: Four-in-one residual plot for biofixation.

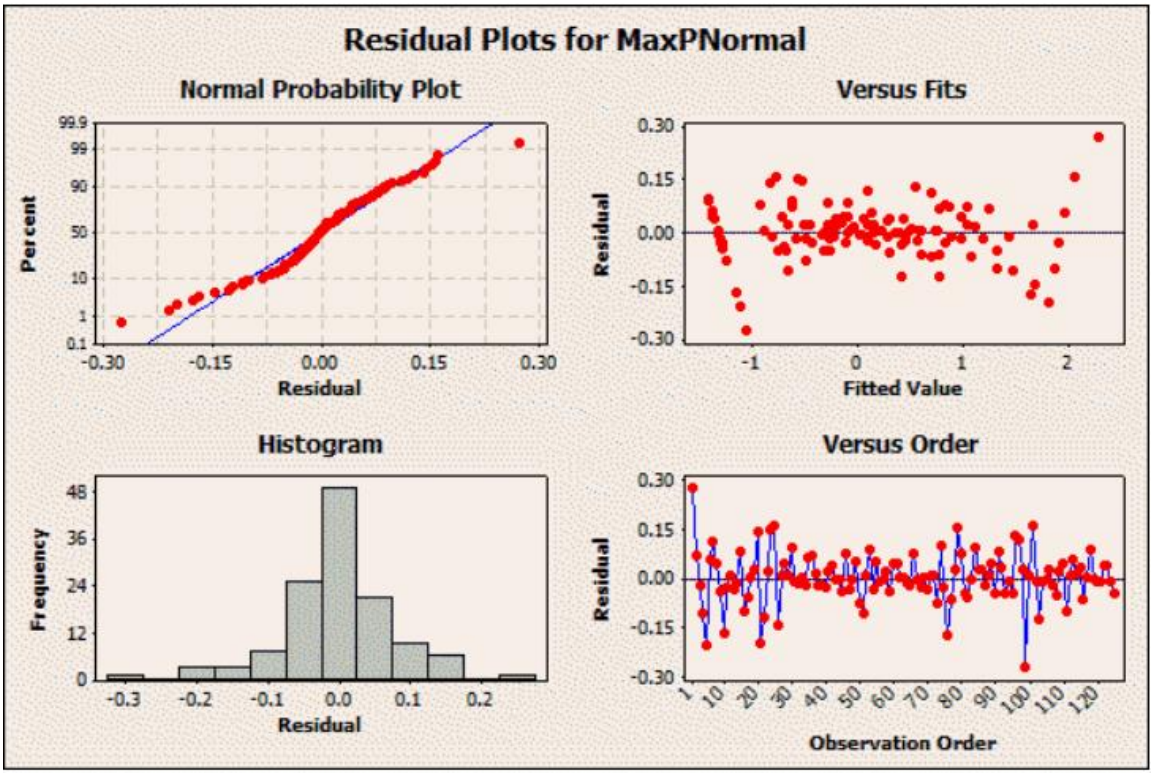


Fig. 8-10: Four-in-one residual plot for maximum biomass productivities.

8.2.4. Analysis of The Single Response Factorial.

From the above Fig. 8-9 and Fig. 8-10, applying the *p*-value approach, it can be observed that all the *p*-values both for the single factors and their interaction have $p < \alpha = 0.01$. Hence in all cases, the null hypotheses can be rejected and conclude that all the single factors and their interactions have significant effect on each of the response variables.

8.3. Nonparametric Analysis for Specific Growth

Considering the outcome of the Nonparametric analysis of specific growth rate. After all effort to normalize the specific growth rate proved abortive, hence only the analysis of the main effects would be presented. For the nonparametric analysis, $\alpha = 0.05$, this quite makes sense because $\alpha = 0.01$ due to the normalization of Maximum biomass productivities which were only able to obtain at $\alpha = 0.01$.

8.3.1. Conclusion

From the results obtained from both MANOVA and the single response Factorial design, it can be concluded that each of the three single factors (i.e. nutrient ratios, culturing temperature and CO_2 concentration) and their interactions have significant effects on all the two normalized responses (i.e. CO_2 biofixation rate and Maximum Biomass Productivities)

CHAPTER 9

CONCLUSION AND RECOMMENDATION

9.1. Conclusion

The effect of CO_2 concentration and microalgae strains on microalgae growth, CO_2 capture rate and efficiency of wastewater treatment have been assessed in this study, in order to obtain an integrated and sustainable biofuel production system. From the results, *C. vulgaris* have the highest CO_2 uptake rate of 150_mg/L/day out of the four microalgae strains investigated. Therefore, optimization of culturing parameters for *Chlorella vulgaris* was further examined. Culturing parameters was optimized using response surface methodology.

Also, *Chlorella vulgaris* exhibited higher CO_2 fixation and biomass productivity as compared to other strains from literature (i.e. *Chlorella kessleri*). Appropriate models of microalgae cultures were developed separately using CCD to maximize specific growth rate, CO_2 fixation rate and biomass productivity of *Chlorella vulgaris.* The model predictions showed good agreement with the actual experimental data. The maximum specific growth rate of 1.93 ± 0.19 d^{-1} was observed at an optimal set of 34ºC, 4:1 NP ratio, and 6% CO_2 concentration. The maximum CO_2 fixation rate of 251.9 ± 13.5 $mgL^{-1}d^{-1}$ was also found at 4% CO_2 concentration, 1:1 NP ratio and $25^{O}C$. Also, 4.8% CO_2 concentration, 8:1 NP ratio and $28^{O}C$ maximized biomass productivity to 86.5 ± 20.0 $mgL^{-1}d^{-1}$. Finally, a multi-objective optimization was done using response optimizer plot to maximize biomass productivity and CO_2 uptake rate simultaneously. The maximum CO_2 uptake rate of 182.84 ± 8.42 mg/L/day and the maximum biomass productivity of 78.5 ± 10.0 $mgL^{-1}d^{-1}$

were obtained at an optimal set of 4% CO_2 concentration, 6:1 NP ratio and 25^{O}C. All the above optimal sets were also validated further by experimental data with the error less than 15.6 %.

The third study examined the effect of culturing conditions (e.g., CO_2 concentration, NP ratio and culture temperature) of *Chlorella vulgaris* on its CO_2 fixation rate and efficiency of wastewater treatment concurrently. The maximum CO_2 fixation rate, nitrogen and phosphorus removal efficiencies were determined to be 127.7 ± 5.1 $mgL^{-1}day^{-1}$, 99.9 ± 7.7 % and 88.5 ± 3.6 %, respectively at an optimal set of 4% CO_2 concentration, 7:1 nutrient ratio and 28^{o}C. Moreover, with the help of ANOVA, R-squared and percentage error, it appeared that the developed regression models can accurately describe the responses. These findings have the potential to be used in the design and scale-up of industrial microalgae cultivation with the aim of CO_2 fixation and generation of biomass for biofuel industries.

9.2. Recommendation

- Response optimizer as a multi-objective optimization method could be used to determine the optimal feeding strategy to maximize CO_2 fixation rate, biomass productivity and wastewater treatment efficiency simultaneously in fed batch and continuous systems.
- The mathematical model of *Chlorella vulgaris* cultivated in either BBM or wastewater in the outdoor closed raceway or laboratory photo bioreactor could also be investigated. As said earlier in chapter 3, low accuracy of the quadratic model for specific growth rate developed was observed which suggest that quadratic models could not probably describe the dynamic behavior of microalgae specific growth rate, biomass productivity and perhaps it CO_2 biofixation in most kinetic regimes. Hence, probably, a mathematical model should be probably investigated in the future.

- The microalgae cultivation can be performed in the raceway photo bioreactor in a continuous mode to provide a basis for the design and scale-up of industrial microalgae cultivation with the aim of CO_2 fixation and generation of biomass for biofuel industries
- The industrial microalgae cultivation can be performed at a larger scale in an integrated fashion by exploiting CO_2 from flue gas, medium from municipal waste water and phosphate and ammonium from agricultural run-off.
- The direct injection of CO_2 into the tailing ponds water can be investigated, since microalgae growth and CO_2 fixation can provide oxygen for aerobic micro-organisms and improve the biodegradation of unwanted dissolved compounds in the ponds.

Appendix

Minitab Sequence 1: Test for equality of variance for specific growth.

```
Test and CI for Two Variances: Experiment, Model

Method

Null hypothesis    Sigma(Experiment) / Sigma(Model) = 1
Alternative hypothesis Sigma(Experiment) / Sigma(Model) not = 1
Significance level      Alpha = 0.05


Statistics

Variable      N  StDev  Variance
Experiment   15  0.695     0.482
Model        15  0.535     0.287

Ratio of standard deviations = 1.297
Ratio of variances = 1.682

95% Confidence Intervals

CI for
Distribution   CI for StDev      Variance
of Data             Ratio            Ratio
Normal        (0.752, 2.238)  (0.565, 5.011)
Continuous    (0.739, 2.609)  (0.546, 6.808)

Tests

Test
Method              DF1  DF2  Statistic  P-Value
F Test (normal)      14   14       1.68    0.342
Levene's Test         1   28       1.17    0.289
(any continuous)
```

Minitab Sequence 2: t-test for specific growth.

```
Two-Sample T-Test and CI: Experiment, Model

Two-sample T for Experiment vs Model

                N    Mean   StDev    SE Mean
Experiment     15   0.842   0.695       0.18
Model          15   0.885   0.535       0.14


Difference = mu (Experiment) - mu (Model)
Estimate for difference:  -0.043
95% CI for difference:  (-0.506, 0.421)
T-Test of difference = 0 (vs not =): T-Value = -0.19
P-Value = 0.852  DF = 28
Both use Pooled StDev = 0.6201
```

Minitab Sequence 3: Minitab output for the 2-sample comparison of BP.

```
Results for: ExpVsModelCompareData.MTW

Mann-Whitney Test and CI: Expt, Model

N  Median
Expt    15    19.30
Model   15    23.35


Point estimate for ETA1-ETA2 is 0.00
95.4 Percent CI for ETA1-ETA2 is (-23.36,20.64)
W = 226.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant ↩
    at 0.8035
The test is significant at 0.8008 (adjusted for ties)
```

Minitab Sequence 4: Minitab output for the 2-sample comparison of Biofixation.

```
1     Results for: ExpVsModelCompareDataBioFix.MTW
2
3     Mann-Whitney Test and CI: Experiment, Model
4
5     N  Median
6     Experiment  15   34.49

7     Model       15   48.79
8
9
10    Point estimate for ETA1-ETA2 is -0.07
11    95.4 Percent CI for ETA1-ETA2 is (-47.66,34.51)
12    W = 227.0
13    Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant ↩
          at 0.8357
14    The test is significant at 0.8347 (adjusted for ties)
```

Minitab Sequence 5: The Manova Analysis for Normalized CO_2 fix and BP.

```
ANOVA: BioFixNormal, MaxPNormal versus C, NR, T

MANOVA for C
s = 2    m = 0.5    n = 30.5

                          Test                  DF
Criterion            Statistic       F  Num   Denom      P
Wilks                  0.06807  44.617    8     126  0.000
Lawley-Hotelling      13.31537 103.194    8     124  0.000
Pillai                 0.95746  14.694    8     128  0.000
Roy                   13.28714


MANOVA for NR
s = 2    m = 0.5    n = 30.5

                          Test                  DF
Criterion            Statistic       F  Num   Denom      P
Wilks                  0.06143  47.796    8     126  0.000
Lawley-Hotelling       6.70840  51.990    8     124  0.000
Pillai                 1.46504  43.817    8     128  0.000
Roy                    4.99146


MANOVA for T
s = 2    m = 0.5    n = 30.5

                          Test                  DF
Criterion            Statistic       F  Num Denom      P
Wilks                  0.00532 200.231    8   126  0.000
```

```
Lawley-Hotelling 165.62682  1283.608    8    124   0.000
Pillai           1.10860    19.898      8    128   0.000
Roy              165.49738

MANOVA for C*NR
s = 2     m = 6.5     n = 30.5

                    Test                DF
Criterion          Statistic      F   Num   Denom       P
Wilks              0.22758    4.316    32     126   0.000
Lawley-Hotelling   2.93860    5.694    32     124   0.000
Pillai             0.87609    3.118    32     128   0.000
Roy                2.77441

MANOVA for C*T
s = 2     m = 6.5     n = 30.5

                    Test                DF
Criterion          Statistic      F   Num   Denom       P
Wilks              0.07908   10.064    32     126   0.000
Lawley-Hotelling   9.24127   17.905    32     124   0.000
Pillai             1.11099    4.999    32     128   0.000
Roy                8.97343


MANOVA for NR*T
s = 2     m = 6.5     n = 30.5

                    Test                DF
Criterion          Statistic      F   Num   Denom       P
Wilks              0.18594    5.194    32     126   0.000
Lawley-Hotelling   3.05881    5.926    32     124   0.000
Pillai             1.05935    4.505    32     128   0.000
Roy                2.53930
```

Minitab Sequence 6: Single response (univariate) Anova output for Biofixation.

```
General Linear Model: BioFixNormal versus C, NR, T

Factor  Type   Levels  Values
C       fixed       5  0, 2, 6, 10, 12
NR      fixed       5  1, 2, 4, 6, 8
T       fixed       5  25, 32, 39, 50, 60

Analysis of Variance for BioFixNormal, using Adjusted SS for Tests

Source    DF     Seq SS     Adj SS    Adj MS         F      P
C          4     6.4250     6.4250    1.6063     87.20  0.000
NR         4     3.9785     3.9785    0.9946     54.00  0.000
T          4   107.2557   107.2557   26.8139   1455.70  0.000
C*NR      16     2.3933     2.3933    0.1496      8.12  0.000
C*T       16     5.6778     5.6778    0.3549     19.27  0.000
NR*T      16     2.3494     2.3494    0.1468      7.97  0.000
Error     64     1.1789     1.1789    0.0184
Total    124   129.2586

S = 0.135720   R-Sq = 99.09%   R-Sq(adj) = 98.23%

Unusual Observations for BioFixNormal

Obs   BioFixNormal       Fit   SE Fit   Residual   St Resid
16         1.82768   2.03299  0.09481   -0.20531      -2.11 R
21         2.90292   2.49419  0.09481    0.40873       4.21 R
45        -1.59000  -1.37753  0.09481   -0.21247      -2.19 R
90        -1.52016  -1.31004  0.09481   -0.21012      -2.16 R
101        2.29035   2.07968  0.09481    0.21067       2.17 R

R denotes an observation with a large standardized residual.
```

Minitab Sequence 7: Single response (univariate) Analysis of Variance for BP.

```
General Linear Model: MaxPNormal versus C, NR, T

Factor  Type   Levels  Values
C       fixed       5  0, 2, 6, 10, 12
NR      fixed       5  1, 2, 4, 6, 8
T       fixed       5  25, 32, 39, 50, 60

Analysis of Variance for MaxPNormal, using Adjusted SS for Tests

Source   DF     Seq SS    Adj SS    Adj MS         F      P
C         4     8.4384    8.4384    2.1096    185.29  0.000
NR        4     1.7067    1.7067    0.4267     37.47  0.000
T         4    92.0804   92.0804   23.0201   2021.90  0.000
C*NR     16     1.2684    1.2684    0.0793      6.96  0.000
C*T      16     5.1807    5.1807    0.3238     28.44  0.000
NR*T     16     1.2439    1.2439    0.0777      6.83  0.000
Error    64     0.7287    0.7287    0.0114
Total   124   110.6471

S = 0.106702   R-Sq = 99.34%   R-Sq(adj) = 98.72%

Unusual Observations for MaxPNormal

Obs   MaxPNormal       Fit   SE Fit  Residual  St Resid
1      2.57652   2.30327  0.07454   0.27325      3.58 R
5     -1.32362  -1.11501  0.07454  -0.20861     -2.73 R
10    -1.32362  -1.15538  0.07454  -0.16824     -2.20 R
21     1.61742   1.81636  0.07454  -0.19894     -2.61 R

25    -0.61747  -0.77389  0.07454   0.15642      2.05 R
76     1.48432   1.66224  0.07454  -0.17792     -2.33 R
99    -1.32362  -1.04837  0.07454  -0.27526     -3.61 R
101    2.22699   2.06732  0.07454   0.15966      2.09 R

R denotes an observation with a large standardized residual.
```

Minitab Sequence 8: Main Effect of CO_2 Conc. on Specific growth rate.

```
Kruskal-Wallis Test: SG versus C

Kruskal-Wallis Test on SG

C          N   Median   Ave Rank      Z
0         25   0.7076       51.1  -1.84
2         25   0.7795       50.7  -1.90
6         25   1.0083       60.5  -0.38
10        25   1.2622       72.3   1.43
12        25   1.5649       80.5   2.69
Overall  125                63.0

H = 13.16  DF = 4  P = 0.011
H = 13.26  DF = 4  P = 0.010  (adjusted for ties)
```

Minitab Sequence 9: Main Effect of NP ratio on Specific Growth rate.

```
Kruskal-Wallis Test: SG versus NR

Kruskal-Wallis Test on SG

NR         N   Median   Ave Rank      Z
1         25   0.9550       65.5   0.38
2         25   0.8612       60.9  -0.32
4         25   0.8373       57.9  -0.79
6         25   0.9486       62.4  -0.09
8         25   1.1020       68.4   0.83
Overall  125                63.0

H = 1.26  DF = 4  P = 0.869
H = 1.27  DF = 4  P = 0.867  (adjusted for ties)
```

Minitab Sequence 10: Main Effect of Temperature on Specific Growth rate.

```
Kruskal-Wallis Test: SG versus T

Kruskal-Wallis Test on SG

T           N       Median  Ave Rank      Z
25         25  1.191928704      86.5   3.63
32         25  1.271000000      94.3   4.83
39         25  1.101996020      82.8   3.06
50         25  0.397989530      38.3  -3.81
60         25  0.000000000      13.1  -7.70
Overall   125                   63.0

H = 95.80  DF = 4  P = 0.000
H = 96.48  DF = 4  P = 0.000  (adjusted for ties)
```

References

Al Ketife, A., Judd, S., Znad, H., 2016. Optimization of cultivation conditions for combined nutrient removal and CO2 fixation in a batch photobioreactor. J. Chem. Technol. Biotechnol. doi:10.1002/jctb.5084

Almeida, M., Erthal, R., Padua, E., Silveira, L., Am, L., 2008. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. Talanta 76, 965–977. doi:10.1016/j.talanta.2008.05.019

Andersen, R., 2013. Algal Culturing Techniques, Phycological Society of America. Amsterdam, Boston, Heiddelberg. doi:10.1017/CBO9781107415324.004

Anjos, M., Fernandes, B.D., Vicente, A.A., Teixeira, J.A., Dragone, G., 2013. Optimization of CO2 bio-mitigation by Chlorella vulgaris. Bioresour. Technol. 139, 149–154. doi:10.1016/j.biortech.2013.04.032

Aslan, N., Cebeci, Y., 2007. Application of Box-Behnken design and response surface methodology for modeling of some Turkish coals. Fuel 86, 90–97. doi:10.1016/j.fuel.2006.06.010

Béchet, Q., Shilton, A., Guieysse, B., 2013. Modeling the effects of light and temperature on algae growth: State of the art and critical assessment for productivity prediction during outdoor cultivation. Biotechnol. Adv. 31, 1648–1663. doi:10.1016/j.biotechadv.2013.08.014

Boonchai, R., Seo, G.T., Park, D.R., Seong, C.Y., 2012. Microalgae Photobioreactor for Nitrogen and Phosphorus Removal from Wastewater of Sewage Treatment Plant. Int. J. Biosci. Biochem. Bioinforma. 2, 0–3.

Boyd, P.W., Rynearson, T., Armstrong, E., Fu, F., Hayashi, K., Hu, Z., Hutchins, D., Kudela, R., Litchman, E., Mulholland, M., Passow, U., Strzepek, R., Whittaker, K., Yu, E., Thomas, M., 2013. Marine Phytoplankton Temperature versus Growth Responses from Polar to Tropical Waters - Outcome of a Scientific Community-Wide Study. PLoS One 8, 1–15. doi:10.1371/journal.pone.0063091

Carvalho, A.P., Silva, S.O., Baptista, J.M., Malcata, F.X., 2011. Light requirements in microalgal photobioreactors: An overview of biophotonic aspects. Appl. Microbiol. Biotechnol. 89, 1275–1288. doi:10.1007/s00253-010-3047-8

Cheng, J., Huang, Y., Feng, J., Sun, J., Zhou, J., Cen, K., 2013. Improving CO2 fixation efficiency by optimizing Chlorella PY-ZU1 culture conditions in sequential bioreactors. Bioresour. Technol. 144, 321–327. doi:10.1016/j.biortech.2013.06.122

Cheng, L., Zhang, L., Chen, H., Gao, C., 2006. Carbon dioxide removal from air by microalgae cultured in a membrane-photobioreactor. Sep. Purif. Technol. 50, 324–329. doi:10.1016/j.seppur.2005.12.006

Chinnasamy, S., Ramakrishnan, B., Bhatnagar, A., Das, K.C., 2009. Biomass production potential of a wastewater alga chlorella vulgaris ARC 1 under elevated levels of CO2 and temperature. Int. J. Mol. Sci. 10, 518–532. doi:10.3390/ijms10020518

Chisti, Y., 2007. Biodiesel from microalgae. Biotechnol. Adv. 25, 294–306. doi:10.1016/j.biotechadv.2007.02.001

Chiu, S., Kao, C., Chen, C., Kuan, T., Ong, S., Lin, C., 2008. Reduction of CO 2 by a high-density culture of Chlorella sp . in a semicontinuous photobioreactor. Bioresour. Technol. 99, 3389–3396. doi:10.1016/j.biortech.2007.08.013

Cho, D.H., Ramanan, R., Heo, J., Lee, J., Kim, B.H., Oh, H.M., Kim, H.S., 2015. Enhancing microalgal biomass productivity by engineering a microalgal-bacterial community. Bioresour. Technol. 175, 578–585. doi:10.1016/j.biortech.2014.10.159

Choi, H.J., Lee, S.M., 2015. Effect of the N/P ratio on biomass productivity and nutrient removal from municipal wastewater. Bioprocess Biosyst. Eng. 38, 761–766. doi:10.1007/s00449-014-1317-z

Choul-gyun, L. and, 2002. Nitrogen Removal from Wastewaters by Microalgae Without Consuming Organic Carbon Sources. J. Microbiol. Biotechnol 12, 979–985.

Converti, A., Casazza, A.A., Ortiz, E.Y., Perego, P., Borghi, M. Del, 2009. Effect of temperature and nitrogen concentration on the growth and lipid content of Nannochloropsis oculata and Chlorella vulgaris for biodiesel production. Chem. Eng. Process. Process Intensif. 48, 1146–1151. doi:10.1016/j.cep.2009.03.006

De Morais, M.G., Costa, J.A. V, 2007. Carbon dioxide fixation by Chlorella kessleri, C. vulgaris, Scenedesmus obliquus and Spirulina sp. cultivated in flasks and vertical tubular photobioreactors. Biotechnol. Lett. 29, 1349–1352. doi:10.1007/s10529-007-9394-6

Duarte, C., 2007. Marine ecology warms up to theory. Trends Ecol. Evol. 22, 331–333.

Fan, L., Zhang, Y., Cheng, L., Zhang, L., Tang, D., Chen, H., 2007. Optimization of carbon dioxide fixation by chlorella vulgaris cultivated in a membrane-photobioreactor. Chem. Eng. Technol. 30, 1094–1099. doi:10.1002/ceat.200700141

Fan, L., Zhang, Y., Zhang, L., Chen, H., 2008. Evaluation of a membrane-sparged helical tubular photobioreactor for carbon dioxide biofixation by Chlorella vulgaris. J. Memb. Sci. 325, 336–345. doi:10.1016/j.memsci.2008.07.044

Farrelly, D.J., Everard, C.D., Fagan, C.C., Mcdonnell, K.P., 2013. Carbon sequestration and the role of biological carbon mitigation : A review. Renew. Sustain. Energy Rev. 21, 712–727.

Feng, P., Deng, Z., Fan, L., Hu, Z., 2012. Lipid accumulation and growth characteristics of Chlorella zofingiensis under different nitrate and phosphate concentrations. J. Biosci. Bioeng. 114, 405–410. doi:10.1016/j.jbiosc.2012.05.007

Feng, Y., Li, C., Zhang, D., 2011. Lipid production of Chlorella vulgaris cultured in artificial wastewater medium. Bioresour. Technol. 102, 101–105. doi:10.1016/j.biortech.2010.06.016

Gao, F., Yang, Z.H., Li, C., Zeng, G.M., Ma, D.H., Zhou, L., 2015. A novel algal biofilm membrane photobioreactor for attached microalgae growth and nutrients removal from secondary effluent. Bioresour. Technol. 179, 8–12. doi:10.1016/j.biortech.2014.11.108

Ghosh, S., Roy, S., Das, D., 2015. Improvement of Biomass Production by Chlorella sp. MJ 11/11 for Use as a Feedstock for Biodiesel. Appl. Biochem. Biotechnol. 175, 3322–3335. doi:10.1007/s12010-015-1503-8

Gonçalves, A.L., Simões, M., Pires, J.C.M., 2014. The effect of light supply on microalgal growth , CO2 uptake and nutrient removal from wastewater. Energy Convers. Manag. 85, 530–536. doi:10.1016/j.enconman.2014.05.085

Gouveia, 2011. Microalgae as a Feedstock for Biofuels. doi:10.1007/978-3-642-17997-6

Hach Company, 2015. Chrmotropic Acid Method, Test "N Tube Vials 10, 1–6.

Hach Company, 2014. Molybdovanadate Method with Acid Persulfate Digestion Test ’N Tube Procedure for Water and Wastewater. Method10127 1–8.

Ho, S.H., Chen, C.Y., Chang, J.S., 2012. Effect of light intensity and nitrogen starvation on CO 2 fixation and lipid/carbohydrate production of an indigenous microalga Scenedesmus obliquus CNW-N. Bioresour. Technol. 113, 244–252. doi:10.1016/j.biortech.2011.11.133

Jacob-Lopes, E., Scoparo, C.H.G., Lacerda, L.M.C.F., Franco, T.T., 2009. Effect of light cycles (night/day) on CO2 fixation and biomass production by microalgae in photobioreactors. Chem. Eng. Process. Process Intensif. 48, 306–310. doi:10.1016/j.cep.2008.04.007

James, M., David, H., Tolbert, E., 1985. Effect of Carbonic Anhydrase Inhibitors on Inorganic Carbon Accumulation by Chlamydomonas reinhardtii ’. Plant Physiol. 79, 177–183.

Ji, M.K., Abou-Shanab, R., Kim, S.H., Salama, E.S., Lee, S.H., Kabra, A.N., Lee, Y.S., Hong, S., Jeon, B.H., 2013. Cultivation of microalgae species in tertiary municipal wastewater supplemented with CO2 for nutrient removal and biomass production. Ecol. Eng. 58, 142–148. doi:10.1016/j.ecoleng.2013.06.020

Jin, H.-F., Lim, B.-R., Lee, K., 2006. Influence of nitrate feeding on carbon dioxide fixation by microalgae. J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng. 41, 2813–2824. doi:10.1080/10934520600967928

Judd, S., van den Broeke, L., Shurair, M., Kuti, Y., Znad, H., 2015. Algal remediation of CO2 and nutrient discharges: A review. Water Res. 87, 356–366. doi:10.1016/j.watres.2015.08.021

Kapdan, I.K., Aslan, S., 2007. Application of the Stover–Kincannon kinetic model to nitrogen removal by Chlorella vulgaris in a continuously operated immobilized photobioreactor system. J. Chem. Technol. Biotechnol. 83, 998–1005. doi:10.1002/jctb

Kasiri, S., 2015. Modeling and Optimization of CO 2 Fixation using Microalgae Cultivated in Oil Sands Process Water. Philos. A thesis Submitt. Partial fulfillment Requir. degree Dr. Phylosophy.

Kasiri, S., Abdulsalam, S., Ulrich, A., Prasad, V., 2015a. Optimization of CO2 fixation by Chlorella kessleri using response surface methodology. Chem. Eng. Sci. 127, 31–39. doi:10.1016/j.ces.2015.01.008

Kasiri, S., Abdulsalam, S., Ulrich, A., Prasad, V., 2015b. Optimization of CO 2 fi xation by Chlorella kessleri using response surface methodology. Chem. Eng. Sci. 127, 31–39. doi:10.1016/j.ces.2015.01.008

Kasiri, S., Ulrich, A., Prasad, V., 2015c. Kinetic Modeling and Optimization of Carbon Dioxide Fixation Using Microalgae Cultivated in Oil-Sands Process Water. Chem. Eng. Sci. 137, 697–711. doi:10.1016/j.ces.2015.07.004

Kevin, K., 2011. The world's most important commodity is often the most overlooked! Money Mark. 1–5.

Kumar, A., Ergas, S., Yuan, X., Sahu, A., Zhang, Q., Dewulf, J., Malcata, X., van Langenhove, H., 2010. Enhanced CO2 fixation and biofuel production via microalgae: Recent developments and future directions. Trends Biotechnol. 28, 371–380. doi:10.1016/j.tibtech.2010.04.004

Lai, J., Yu, Z., Song, X., Cao, X., Han, X., 2011. Journal of Experimental Marine Biology and Ecology Responses of the growth and biochemical composition of Prorocentrum donghaiense to different nitrogen and phosphorus concentrations. J. Exp. Mar. Bio. Ecol. 405, 6–17. doi:10.1016/j.jembe.2011.05.010

Lau P.S., Tam N.F.Y., Wong Y.S., 1995. Effect of Algal Density on Nutrient Removal from Primary Setteled Wastwater. Environ. Sci. Pollut. Reserv. 89, 59–66.

Lau, Tam, Wong, 1996. Wastewater nutrients removal by Chlorella vulgaris: optimization through acclimation. Environ. Technol. 17, 183–189. doi:10.1080/09593331708616375

Li, Y., Horsman, M., Wu, N., Dubois-calero, N., 2008. Biocatalysts and Bioreactor Design. Biotechnol. Program. 24, 815–820. doi:10.1021/bp.070371k

Liang, Y., Sarkany, Æ.N., Cui, Æ.Y., 2009. Biomass and lipid productivities of Chlorella vulgaris under autotrophic , heterotrophic and mixotrophic growth conditions. Biotechnol. Lett. 31, 1043–1049. doi:10.1007/s10529-009-9975-7

Lundguist T.J., Woertz I.C, Quinn N.W.T, Benemann J.R., 2010. A Realistic Technology and Engineering Assessment of Algae Biofuel Production. Energy Biosci. Inst. 3–9.

Lv, J.M., Cheng, L.H., Xu, X.H., Zhang, L., Chen, H.L., 2010. Enhanced lipid production of Chlorella vulgaris by adjustment of cultivation conditions. Bioresour. Technol. 101, 6797–6804. doi:10.1016/j.biortech.2010.03.120

Maher, C., Neethling, Murthy, S., Pagilla, K., 2015. Kinetics and capacities of phosphorus sorption to tertiary stage wastewater alum solids, and process implications for achieving low-level phosphorus effluents. Water Res. 85, 226–234. doi:10.1016/j.watres.2015.08.025

Mata, T.M., Cardoso, N., Ornelas, M., Neves, S., Caetano, N.S., 2011. Evaluation of two purification methods of biodiesel from beef tallow, pork lard, and chicken fat. Energy and Fuels 25, 4756–4762. doi:10.1021/ef2010207

Mata, T.M., Melo, A.C., Meireles, S., Mendes, A.M., Martins, A.A., Caetano, N.S., 2013. Potential

of microalgae scenedesmus obliquus grown in brewery wastewater for biodiesel production. Chem. Eng. Trans. 32, 901–906. doi:10.3303/CET1332151

Mathews, J., 2008. Carbon-negative biofuels. Energy Policy 36, 940–945. doi:10.1016/j.enpol.2007.11.029

Mauna, L., 2016. Recent Global CO 2. Glob. Monit. Div. 2–5.

Miyachi, S., Iwasaki, I., Shiraiwa, Y., 2003. Historical perspective on microalgal and cyanobacterial acclimation to low- and extremely high-CO 2 conditions. Photosynth. Res. 77, 139–153.

Morais, M.G. De, Alberto, J., Costa, V., 2007. Biofixation of carbon dioxide by Spirulina sp . and Scenedesmus obliquus cultivated in a three-stage serial tubular photobioreactor. J. Biotechnol. 129, 439–445. doi:10.1016/j.jbiotec.2007.01.009

Moroney, J. V., Somanchi, a., 1999. How Do Algae Concentrate CO2 to Increase the Efficiency of Photosynthetic Carbon Fixation? Plant Physiol. 119, 9–16. doi:10.1104/pp.119.1.9

Moroney, J. V, Somanchi, A., 1999. How Do Algae Concentrate CO 2 to Increase the Efficiency of Photosynthetic Carbon Fixation ? 1. Plant Physiol. 119, 9–16.

Mun, R., Guieysse, B., 2006. Algal – bacterial processes for the treatment of hazardous contaminants : A review. Water Reseacrh 40, 2799–2815. doi:10.1016/j.watres.2006.06.011

Nobutaka, H., Takeuchi, T., Fukuju, Y., Barnes, D., Karube, I., 1992. Tolerance of Microalgae to High CO2 and High Temperature. Phytochemistry 31, 3345–3348.

Ogunnaike, B.., 2010. Random phenomena: fundamentals of probability and statistics for Engineers.

Ono, E., 2001. Selection of optimal microalgae species for CO 2 sequestration 1–7.

Pires, J.C.M., Alvim-Ferraz, M.C.M., Martins, F.G., Sim??es, M., 2012. Carbon dioxide capture from flue gases using microalgae: Engineering aspects and biorefinery concept. Renew. Sustain. Energy Rev. 16, 3043–3053. doi:10.1016/j.rser.2012.02.055

Pires, J.C.M., Martins, F.G., 2014. Effect of light supply on CO 2 capture from atmosphere by Chlorella vulgaris and Pseudokirchneriella subcapitata. Mitig. Adapt. Strateg. Glob. Chang. 19, 1109–1117. doi:10.1007/s11027-013-9463-1

Purba, E., Taharuddin, 2010. CO2 reduction and production of algal oil using microalgae Nannochloropsis oculata and Tetraselmis chuii. Chem. Eng. Trans. 21, 397–402. doi:10.3303/CET1021067

Raven, J., Geider, R., 1988. Temperature and algal growth. New Phytol. 110, 441–461. doi:10.1111/j.1469-8137.1988.tb00282.x

Razzak, S., Hossain, M., Lucky, R., Bassi, A., De Lasa, H., 2013. Integrated CO2 capture, wastewater treatment and biofuel production by microalgae culturing - A review. Renew. Sustain. Energy Rev. 27, 622–653. doi:10.1016/j.rser.2013.05.063

Redfield, 1963. The Influence of Organisms on the Composition of Sea Water. Sea 2, 26–77.

Rhodes, J.S., Keith, D.W., 2008. Biomass with capture : negative emissions within social and environmental constraints : an editorial comment. Clim. Chang. 87, 321–328. doi:10.1007/s10584-007-9387-4

Ruiz-Martinez, Garcia, M., Romero, Seco, Ferrer, 2012. Microalgae cultivation in wastewater: Nutrient removal from anaerobic membrane bioreactor effluent. Bioresour. Technol. 126, 247–253. doi:10.1016/j.biortech.2012.09.022

Sakai, N., Sakamoto, Y., Kishimoto, N., Chihara, M., Karube, I., 1995. 0196-8904(95)00100-x. Energy Cover. Manag. 36, 693–696.

Sato, N., Tsuzuki, M., Kawaguchi, A., 2003. Glycerolipid synthesis in Chlorella kessleri 11 h - II. Effect of the CO2 concentration during growth. Biochim. Biophys. Acta - Mol. Cell Biol. Lipids 1633, 35–42. doi:10.1016/S1388-1981(03)00070-2

Sialve, B., Bernet, N., Bernard, O., 2009. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. Biotechnol. Adv. 27, 409–416. doi:10.1016/j.biotechadv.2009.03.001

Silva-benavides, A.M., Torzillo, G., 2012. Nitrogen and phosphorus removal through laboratory batch cultures of microalga Chlorella vulgaris and cyanobacterium Planktothrix isothrix grown as monoalgal and as co-cultures. J. Appl. Phycol. 24, 267–276. doi:10.1007/s10811-011-9675-2

Singh, N.K., Dhar, D.W., 2011. Microalgae as second generation biofuel. A review. Agron. Sustain. Dev. 31, 605–629. doi:10.1007/s13593-011-0018-0

Skorupskaite, V., Makareviciene, V., Levisauskas, D., 2015. Optimization of mixotrophic cultivation of microalgae Chlorella sp . for biofuel production using response surface methodology. Algae Res. 7, 45–50.

Sobczuk, T.M., Garcı, F., Rubio, F.C., 2000. Carbon Dioxide Uptake Efficiency by Outdoor Microalgal Cultures in Tubular Airlift Photobioreactors. John Wiley Sons, Inc.

Spalding, M.H., 2008. Microalgal carbon-dioxide-concentrating mechanisms: Chlamydomonas inorganic carbon transporters. J. Exp. Bot. 59, 1463–1473. doi:10.1093/jxb/erm128

Stepan, D.J., Shockey, R.E., Moe, T. a, Dorn, R., 2002. Subtask 2 . 3 – Carbon Dioxide Sequestering Using Microalgal 32. doi:10.2172/882000

Su, Y., Mennerich, A., Urban, B., 2012. Coupled nutrient removal and biomass production with mixed algal culture: Impact of biotic and abiotic factors. Bioresour. Technol. 118, 469–476. doi:10.1016/j.biortech.2012.05.093

Sun, X., Cao, Y., Xu, H., Liu, Y., Sun, J., Qiao, D., Cao, Y., 2014. Bioresource Technology Effect of nitrogen-starvation , light intensity and iron on triacylglyceride / carbohydrate production and fatty acid profile of Neochloris oleoabundans HK-129 by a two-stage process. Bioresour. Technol. 155, 204–212. doi:10.1016/j.biortech.2013.12.109

Toktas, G., Toktas, A., Karaoglan, A.D., 2014. Mathematical Modeling of Carbon Content and Intercritical Annealing Temperature in DP Steels by Factorial Design Method. J. Iron Steel Res. Int. 21, 715–722.

Toutenburg, H., Shalabh, 2009. Statistical Analysis of Designed Experiments, 3rd ed. Springer US, Kanpur India.

Tredici, M.R., 2016. Photobiology of microalgae mass cultures : understanding the tools for the next green revolution. Biofuels 1, 1–6.

Wang, L., 2006a. Recommendations for Design Parameters for Central Composite Designs with Restricted Randomization.

Wang, L., 2006b. Recommendations for Design Parameters for Central Composite Designs with Restricted Randomization. Diss. Submitt. to Fac. Virginia Polytech. Inst. State Univ. Partial fulfillment Requir. degree Dr. Philos. Stat. 20–60.

Xia, J., Gao, K., 2005. Impacts of Elevated CO 2 Concentration on Biochemical Composition , Carbonic Anhydrase , and Nitrate Reductase Activity of Freshwater Green Algae. J. Integr. Plant Biol. 47, 668–675.

Xin, L., Hong-ying, H., Ke, G., Jia, Y., 2010. Growth and nutrient removal properties of a freshwater microalga Scenedesmus sp . LX1 under different kinds of nitrogen sources. Ecol. Eng. 36, 379–381. doi:10.1016/j.ecoleng.2009.11.003

Xu, Z., Zou, D., Gao, K., 2010. Effects of elevated CO2 and phosphorus supply on growth, photosynthesis and nutrient uptake in the marine macroalga Gracilaria lemaneiformis (Rhodophyta). Bot. Mar. 53, 123–129. doi:10.1515/BOT.2010.012

Yeh, K.L., Chang, J.S., 2012. Effects of cultivation conditions and media composition on cell growth and lipid productivity of indigenous microalga Chlorella vulgaris ESP-31. Bioresour. Technol. 105, 120–127. doi:10.1016/j.biortech.2011.11.103

Yewalkar, S., Li, B., Posarac, D., Duff, S., 2011. Potential for CO 2 Fixation by Chlorella pyrenoidosa Grown in Oil Sands Tailings Water. Energy 1900–1905. doi:10.1021/ef101503h

Yr, E.R.H.L., Xli, I., Exmsr, G., Rsfpi, S.J., Tvshygxw, E., Xli, M., Higehi, R.I.X., 2003. Photobioreactor Engineering: Design and Performance. Biotechnollogy Bioprocess Eng. 8, 313–321.

Yuan, T., Tahmasebi, A., Yu, J., 2015. Comparative study on pyrolysis of lignocellulosic and algal biomass using a thermogravimetric and a fixed-bed reactor. Bioresour. Technol. 175, 333–341. doi:10.1016/j.biortech.2014.10.108

Zheng, Y., Chen, Z. a, Lu, H.B., Zhang, W., 2011. Optimization of carbon dioxide fixation and starch accumulation by Tetraselmis subcordiformis in a rectangular airlift photobioreactor. African J. Biotechnol. 10, 1888–1901. doi:10.5897/AJB10.1620

Zhu, L., Wang, Z., Shu, Q., Takala, J., Hiltunen, E., Feng, P., Yuan, Z., 2013. Nutrient removal and biodiesel production by integration of freshwater algae cultivation with piggery

wastewater treatment. Water Reseacrh 7, 4294–4302.

Zimmerman, R., Faris, C., 2011. Climate change mitigation and adaptation in North American cities. Environ. Sustain. 3, 181–187. doi:10.1016/j.cosust.2010.12.004

Vitae

Name	Mohammed Kazeem Ayodeji
Nationality	Nigerian
Date of Birth	9/27/1987
Email	Mohammed.kazeem@udusok.edu.ng
Address	4 Obangede Street, Adewole Estate, Ilorin, Kwara state, Nigeria. Poster address: 240243.
Academic Background	• BSc Applied Chemistry, Usmanu Dan Fodio University, Sokoto. • M.Sc. Chemical Engineering, King Fahd University of Petroleum and Minerals.
Awards & Honors:	• Certificate of Excellence (Best Organic Chemistry student and overall best second year student), • Top two graduating student (Pure & Applied Chemistry department). • Nigerian Federal Government Scholarship • Scholarship for MSc. degree at King Fahd University of Petroleum and Minerals, Saudi Arabia.
Research output	• **M.A. Kazeem**, S.M.H. Hossain, M.M. Hossain and S.A Razzak, (2018). "*The Application of Central Composite Design to Optimize Culture Conditions of Chlorella vulgaris for CO_2 fixation in a Batch Photobioreactor"*. Chemical Product and Process Modeling. **Accepted** • U. Zahid, F.N. Al Rowaili, **K.A. Mohammed** and U. Ahmed, (2017). *"Performance Analysis of Industrial CO_2 capture from Natural Gas Using Diglycolamine"*. Presented at 27th European Symposium on Computer Aided Process Engineering, Page 2641. **Published** • U. Zahid, F.N. Al Rowaili, **K.A. Mohammed** and U. Ahmed, (2016). "*Simulation and Parametric Analysis of CO_2 Capture from Natural Gas using Diglycolamine"*. International Journal of Greenhouse Gas Control. Vol. 57, Pg. 42-51. **Published**